JOURNAL OF DAIRY SCIENCE

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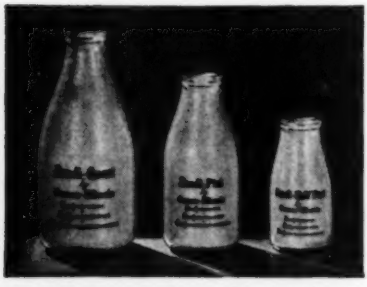

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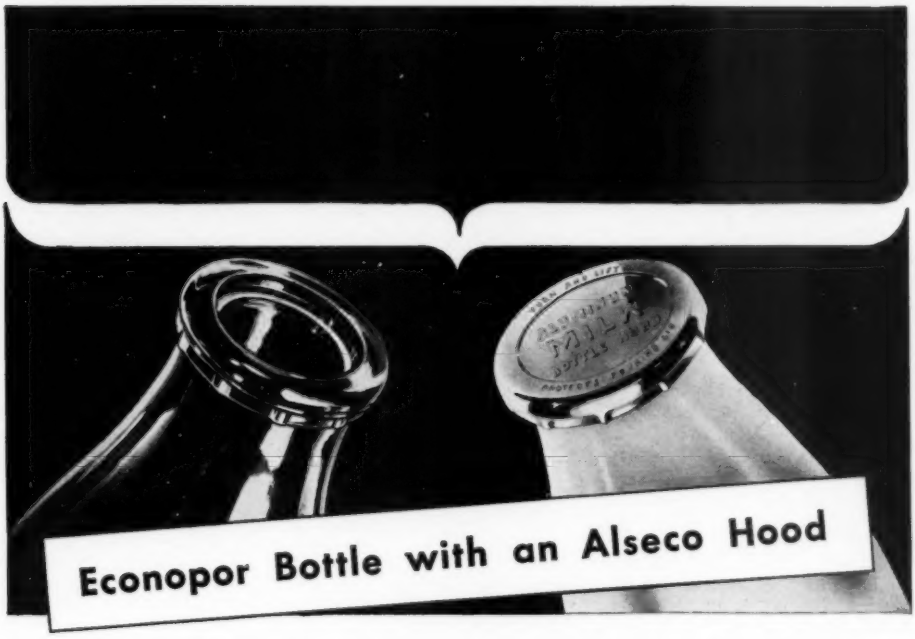

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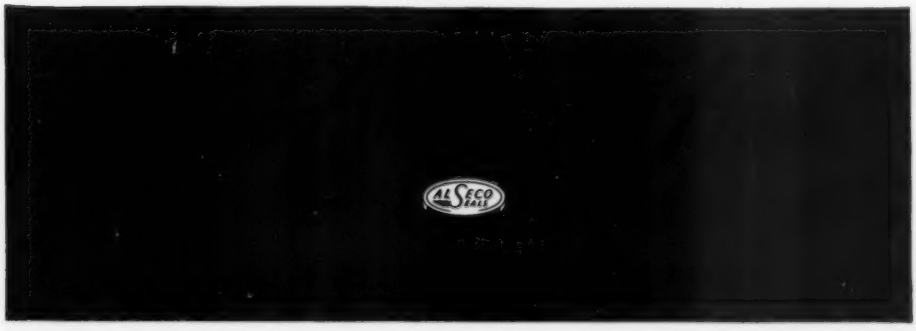

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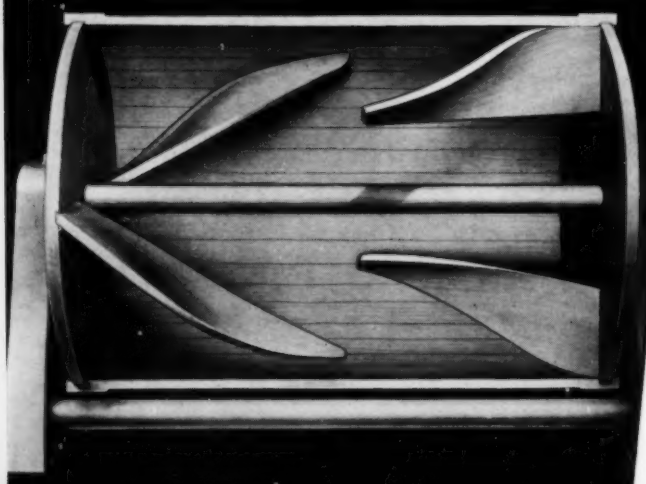

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September 10, 1937

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Gentlemen:

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Tub No.	Butterfat	Fat-Curd	Per Cent Water	Per Cent Salt
1	80.2	.8	14.0	2.8
2	80.3	.8	14.1	2.8
3	80.2	.8	14.0	2.8
4	80.2	.8	14.0	2.8
5	80.3	.8	14.1	2.8
6	80.2	.8	14.0	2.8
7	80.3	.8	14.0	2.8
8	80.2	.8	14.0	2.8
9	80.3	.8	14.1	2.8
10	80.2	.8	14.0	2.8
11	80.2	.8	14.0	2.8
12	80.3	.8	14.1	2.8
13	80.2	.8	14.0	2.8
14	80.2	.8	14.0	2.8
15	80.2	.8	14.0	2.8
16	80.1	.8	14.0	2.8
17	80.2	.8	14.0	2.8
18	80.2	.8	14.1	2.8
Average	80.2	.8	14.0	2.8

These tests were made by the Babcock Method.

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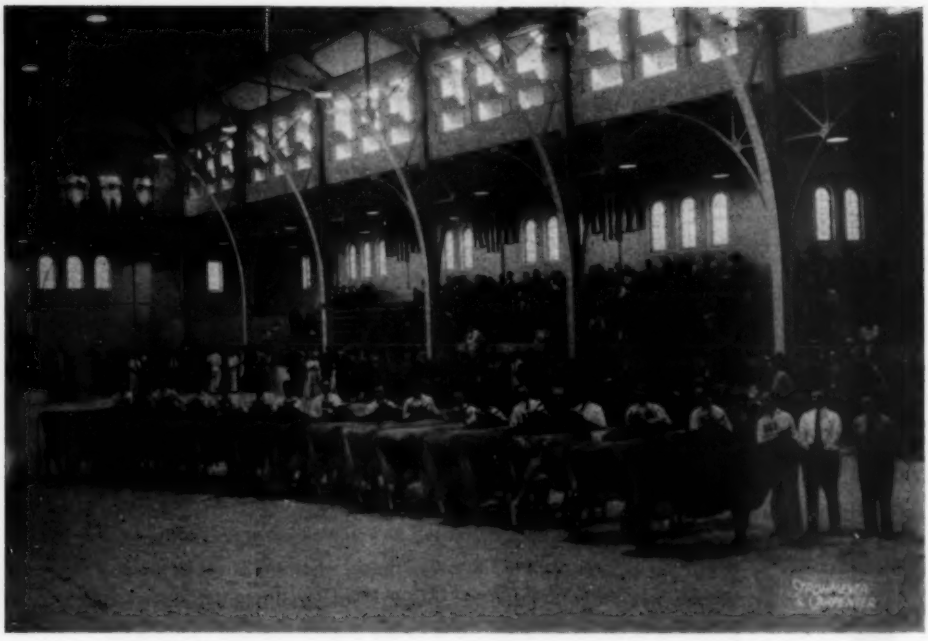

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JOURNAL OF DAIRY SCIENCE

VOLUME XXIX FEBRUARY, 1946 NUMBER 2

THE OCCURRENCE AND SURVIVAL OF BRUCELLA ABORTUS IN CHEDDAR AND LIMBURGER CHEESE*

H. L. GILMAN
College of Veterinary Medicine, Cornell University, Ithaca, N. Y.

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Department of Agriculture and Markets, Albany, N. Y.

Most dairy products reach consumers after being subjected to a heat treatment sufficient to destroy any pathogenic bacteria that might be present, but there is still much unpasteurized Cheddar cheese made from raw milk. Since such cheese has not been found to cause undulant fever in man, one might reasonably expect that *Brucella abortus* dies or becomes non-infectious during normal cheese curing. It is known that the dairy cows of the United States are often infected and that infected cows often give milk containing these bacteria. Conditions in the early stages of the manufacture of Cheddar cheese favor bacterial development and the bacteria are concentrated in the fresh curd. Some of the cheese might be infected so the question of the survival of *Brucella abortus* in Cheddar cheese has public health significance.

This study was undertaken to show the length of time that *Brucella abortus* survives in Cheddar cheese. First a preliminary survey was made on cheese milk in New York State. Then a study was conducted on cheese made from milk inoculated with cultures of the organism recently isolated from naturally infected milk. Tests were made on cheese manufactured from milk from selected reacting cows that were found by test to be producing milk heavily infected with *Brucella abortus*. Finally, a survey was conducted showing the contamination naturally occurring in milk of cheese-producing areas in New York and Wisconsin, where brucellosis had not been eradicated from the dairy herds and the survival of *Brucella abortus* in naturally infected commercial Cheddar cheese was determined.

LITERATURE

The literature contains comparatively little information on the viability of *Brucella abortus* in cheese. There is no evidence that undulant fever has

Received for publication September 6, 1945.

* This investigation was aided by a grant from the National Cheese Institute and was originally proposed by its research committee.

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been contracted as a result of eating cheese made solely from cow's milk. On the other hand, cheese made from infected, unpasteurized goat's milk has been found to be highly infective for man, especially when consumed soon after manufacture. As with most bacteria, the survival of Brucella in milk and milk products is influenced greatly by such factors as duration of storage, temperature, and acidity.

Eyre (6) isolated *Brucella melitensis* from goat's cheese up to 48 hours after manufacture. After this time, he states that recovery was impossible due to the increased growth of lactic acid bacteria. Versilova (16) found that *Brucella melitensis* remained viable and infective for guinea pigs for 45 days at 11° to 14° C. in Brynza cheese prepared from milk inoculated with *Brucella melitensis* after pasteurization. In sheep's milk with a pH of 6.8 to 6.0 *Brucella melitensis* was viable 22 to 40 days at a temperature of 11° C. At pH 5.0 to 4.0 it remained viable 15 days, but never more than 30 days, whereas in milk kept at 37° C. it frequently died the first few days.

Peres and Granon-Fabre (10) report the recovery by guinea-pig inoculation of *Brucella melitensis* from cheese made from naturally infected goat's milk which had been maturing for 20 days, but not from the samples maturing 6, 10, or 17 days. Veloppe and Jaubert (15) report 14 cases of undulant fever due to fresh cheese. There were two deaths. They emphasize that it is common practice to mix the milk of goats, sheep, and cattle. The authors conclude that infected cheese is harmless after 30 days.

Carpenter and Boak (4) injected, into guinea pigs, 82 samples of cheese of various kinds, 72 of which were imported. Of the 72 imported samples, 30 were manufactured in Italy, 14 in France, 13 in Switzerland, 6 in Holland, and the remainder in England, Germany, and North and South America. The majority of the types examined were Swiss, Roquefort, Reggiano, Gorgonzola, and Edam cheeses. There was no indication as to the age of any of the foreign or domestic types. Infection with *Brucella abortus* or *Brucella melitensis* was not found in any of the guinea pigs injected with these samples.

Smith (11) examined samples of Cheshire, Cheddar (red and white), Double Gloucester, Bel Paese, Convalli, Camembert, Wenslet, Roquefort, Gouda, Stilton, Edam, Gorgonzola, Chilvern, Parmesan, and locally prepared Scotch cheese. Twenty-three of the cheeses were made in England, 6 were colonial in origin, 28 foreign, and 6 local. The samples were prepared by passing the cheese through a fine tissue mincer, thereafter adding saline to make a fine paste, and then injected in 5-ml. amounts into each of two guinea pigs. Examination of all 63 samples failed to show the presence of *Brucella abortus* or *Brucella melitensis*.

Stiles (12) examined 19 cheese samples, of which 8 were listed as positive for *Brucella melitensis* when injected into guinea pigs. Four samples were of the Feta type made from raw goat's milk, 3 of the Romano type made

from raw goat's milk, and one was imported (Mexico) yellow cream cheese from cow's milk. It is quite possible that this cheese was made from a combination of goat's and cow's milk in view of the fact that it is common practice in some countries to combine any types of milk available. The reaction of the cheeses was given as pH 6.8. The "probable age" of the positive cheeses at the time of study was from 38 to 100 days. One cheese made from cow's and goat's milk was listed as positive after one year. However, the positive findings in this case are open to question inasmuch as *Brucella melitensis* was not recovered from the inoculated guinea pig. Lesions and cultures were negative. The blood titer of 1:200 in this case could have been caused by a large number of dead Brucella organisms present in the cheese. In a footnote, the author states that 6 additional samples of Colorado goat cheese of the Romano (Incannestrato) type had been examined since the preparation of the paper. Of this number, 4 were negative by guinea pig tests, and 2 proved positive. The 2 positive samples were from lots of cheese manufactured 64 and 70 days prior to the examination. The guinea pigs showed typical lesions, and yielded pure cultures of *Brucella melitensis*.

Lerche (8) states that the viability of *Brucella abortus* in cheese is greatly dependent on the acidity of the product. In all cheese, such as Landekäse, made by the natural souring of milk, the organism dies rapidly. However, in milk artificially infected with *Brucella abortus* and made into Weisskäse and Frühstückkäse by the rennet process, it persisted up to 24 days. Drescher and Hopfengartner (5) made up several types of cheeses from milk that had been heavily inoculated with *Brucella abortus*. At intervals, samples of cheese from near the rind and from the inner portion were taken for guinea-pig inoculations and cultures. One gram of cheese, plus 1 ml. of physiological saline solution were inoculated intraperitoneally in amounts of 0.5, 1, 2, and 3 ml. in each guinea pig. In Delikatkäse the organism was recovered from the rind and inner portion at 35, but not at 42 days. The cheese had been stored at 4° to 5° C. In Tilsiterkäse the organism lived up to 35 days as determined by culture and guinea-pig inoculation (49 days by inoculation only) in the rind, and 91 days by each method in the inner portion of the cheese. In Emmentalekäse *Brucella abortus* survived 49 days in both rind and inner portion. Lichtenstein (9) calls attention to the effect of acidity and temperature on the survival of *Brucella abortus* in milk and milk products. Thompson (14) quotes Voille to the effect that in cheese, especially Roquefort, *Brucella abortus* can remain viable for a period of two months. In view of the fact that Roquefort cheese is made from ewe's milk, it is probable that this organism was of the *melitensis* rather than the *abortus* variety. There is no indication as to whether the cheese was made from naturally infected milk, or from milk heavily inoculated with the organism, as is so frequently done in experimental work.

EXPERIMENTAL

In the summer of 1942 samples of milk were collected at random in the two principal cheese-producing areas of New York State to ascertain the prevalence of *Brucella abortus* in commercial cheese milk. The milk samples were taken from the cheese vats in the factories just prior to adding rennet. They were iced and delivered by car directly to the research laboratory for examination. Samples of cheese from each vat were marked for later identification in case the milk was positive so that the cheese could be tested later for Brucella. Both Cheddar and Limburger cheese factory milks were taken for the study. No consideration was given to the presence of brucellosis in the herds producing milk for the cheese factories. The data on Cheddar cheese are reported as experiment 1, on Limburger cheese as experiment 2.

Milk was artificially inoculated with *Brucella abortus* to give a heavily infected milk. A local dairy herd in which practically all cows reacted was found to be eliminating Brucella in sufficient numbers to give positive results with guinea pigs and a plate count of about 500 per ml. *Brucella abortus* was isolated from this milk and enough of the culture was added to the naturally infected milk to give a total count of approximately 1000 *Brucella abortus* per ml. This milk, in the early spring of 1942, was made into Cheddar cheese on three different days. Each batch of milk was tempered to 30° C., inoculated with 1 per cent of good lactic starter and the *Brucella abortus* culture. The first batch of milk (B.A. 1) was incubated for 30 minutes before adding rennet and the acidity developed slowly to 0.35 per cent in the whey at milling time even though the time from adding culture to milling was 6 hours. This is a low acid cheese. The second batch of milk (B.A. 11) was handled like the first but the acidity developed normally and was 0.56 per cent in the whey when the curd was milled. The B.A. 21 or third batch of milk was incubated with lactic starter and Brucella culture for one hour at 30° C. before rennet was added. The whey acidity at milling was 0.60 per cent. This cheese was well developed in acidity. The standard Cheddar cheese process was employed with a cooking temperature of 37.8° C. The cheeses were cured at 4.4° C. because a low temperature favors survival for long periods, as shown by Campbell and Gibbard (3) for *Eberthella typhosa*, and Yale and Marquardt (18) for *Streptococcus pyogenes*.

Tests were made on the original raw milk, the milk inoculated with *Brucella abortus*, the fresh curd as taken from the vat after salting, and the cheese when one week, two weeks, one month, three months, six months, and one year old. The data are reported as experiment 3.

Cheddar cheese was also manufactured in the laboratory from uninoculated milk that contained the heaviest natural contamination with *Brucella abortus* that was found by selecting milk from individual cows. In the fall of 1942 tests were made on the milk produced by about 30 reacting cows,

and two cows were selected because they were giving large numbers of Brucella in their milk. The *Brucella abortus* plate counts were 700 to 800 per ml. Plate counts of Brucella in milk were made according to the method of Huddleson (7). The milk from the two selected cows was accumulated for two days and then made into cheese. This was repeated on three different days. The methods of manufacturing the cheese were the same as those previously employed. The milks for the three batches were designated as B.A. 31, B.A. 41, and B.A. 51. The raw milk, curd, and cheese were tested for viable Brucella as in the previous test. The data are reported as experiment 4.

Finally, in the early months of 1944 a survey was made of the prevalence of *Brucella abortus* in cheese milk and Cheddar cheese under commercial conditions in areas in Wisconsin and New York where brucellosis is prevalent. It is known that brucellosis has been eradicated from some areas and there is little probability of milk being contaminated with *Brucella abortus*. Milk from such areas was avoided. Factories were chosen whose patrons were known to have made little or no effort to test their cows for brucellosis. After the cheese vat contained the entire quantity of milk to be made into cheese, the milk was stirred and a sample placed into a bottle containing sufficient boric acid powder to make a 0.5 per cent solution in the milk. The boric acid inhibits the growth of bacteria and delays the souring of the milk, but does not kill *Brucella abortus* in this concentration. The milk sample was cooled. In the afternoon a sample of the salted cheese curd was placed in another bottle. Both samples were then sent to the laboratory by special delivery air mail or by automobile. One cheese from each batch was marked to be saved if wanted later for testing in case the milk proved positive for *Brucella abortus*. The cheese was held at the factory for a few days and was then stored in the commercial warehouse at the usual temperature of 1.1° to 2.8° C. At the end of five weeks when the results on the milk were available, the cheeses made from positive milks were secured for further analysis. All tests on the cheeses were made as soon as secured, but this time varied from 41 to 84 days. The cheeses were tested a second time after a lapse of 30 to 60 days. The plan was to test the cheeses when two and four months old. The data are reported as experiment 5.

When milk was received at the laboratory it was placed in the refrigerator overnight to permit creaming. In experiments 1, 2, and 5, four guinea pigs were each injected subcutaneously with 5 ml. of the gravity cream and after five weeks the guinea pigs were killed and examined for Brucella infection. In experiments 3 and 4, eight guinea pigs were used for each milk sample. The cheese was prepared by taking a total of 50 grams from at least three different places in the cheese for experiments 3 and 4, whereas 25 grams were used in experiment 5. The cheese was placed in a sterilized Waring Blendor with 100 ml. of sterile physiological saline solution and

agitated for ten minutes to produce a smooth creamy mixture. The mixture was then filtered through one layer of cheesecloth and 2-ml. amounts were injected subcutaneously into each of the four or eight guinea pigs. Each guinea pig received the equivalent of about 0.8 gram of the laboratory-made cheese and about 0.42 gram of the commercial cheese. The smaller dose of the commercial cheese was the maximum amount that the guinea pigs could tolerate.

Positive tests were those in which the guinea pigs, five weeks after injection, gave positive blood agglutination reactions, characteristic lesions in the slaughtered animals, and recovery of *Brucella abortus* from the spleen. Inasmuch as dead *Brucella abortus* cause a positive blood agglutination test, no animal was regarded as positive unless the organism had been recovered from the spleen by bacteriological culture methods.

RESULTS

Experiment No. 1. Brucella abortus in Milk and Cheddar Cheese from Commercial Cheese Factories Selected at Random

Milk samples were collected from 15 different vats at 11 different commercial Cheddar cheese factories in New York State. Much to our surprise, all 15 milk samples failed to show the presence of *Brucella abortus* as determined by animal inoculation. The organisms were not viable, or had been so greatly diluted in the pooled milk that their presence could not be demonstrated by the method used. The cheeses were therefore released.

Experiment No. 2. Brucella abortus in Milk and Limburger Cheese from Commercial Cheese Factories Selected at Random

Ten milk samples were obtained from 10 different factories making Limburger cheese in New York State. The fresh curd (24 hours old), made from four of these vat samples, was also obtained and injected into guinea pigs. Eight of the 10 milk samples were negative for the presence of *Brucella abortus*. Two milks contained the organism in sufficient quantity to cause infection in the inoculated guinea pigs. The 24-hour-old curd made from these two positive milk samples failed to show the presence of the organism. Samples from these two cheeses were later injected into guinea pigs after storage for 57 days. *Brucella abortus* could not be demonstrated.

Experiment No. 3. Brucella abortus in Cheddar Cheese Manufactured from Milk from a Highly Infected Herd, and Artificially Inoculated with Brucella abortus

This experiment was initiated in an effort to determine the viability of *Brucella abortus* in Cheddar cheese made up under laboratory conditions from milk obtained from an infected herd in which some of the animals were known to be eliminating the organism in the milk. The milk, as deter-

mined by bacteriological count, contained approximately 500 viable *Brucella abortus* per ml. Just before the milk was manufactured into cheese, enough viable *Brucella abortus* culture was added to bring the total Brucella count to approximately 1,000 organisms per ml. The plan was to determine by guinea pig inoculation the presence of viable Brucella in fresh cheese curds, and in cheeses made from inoculated milks stored for one week, two weeks, one month, three months, six months, and one year, respectively.

In series B.A. 1–9 the organism was fully viable in the raw milk, fortified milk, fresh cheese curd, and the stored cheeses up to and including three months. At six months, all guinea pigs were positive but the lesions were slight, showing that they had received comparatively few viable organisms. At one year, all the guinea pigs were negative, as shown in table 1.

TABLE 1

Survival of Brucella abortus in Cheddar cheese manufactured from milk from a highly infected herd, and fortified with Brucella abortus (Exper. No. 3)

Product and age	First series		Second series		Third series	
	Sample	Results	Sample	Results	Sample	Results
Raw milk	B.A. 1	+	B.A. 11	+	B.A. 21	+
Raw milk plus *Brucella abortus*	B.A. 2	+	B.A. 12	+	B.A. 22	+
Fresh curd	B.A. 3	+	B.A. 13	+	B.A. 23	+
Cheese, 1 week	B.A. 4	+	B.A. 14	+	B.A. 24	+
Cheese, 2 weeks	B.A. 5	+	B.A. 15	+	B.A. 25	+
Cheese, 1 month	B.A. 6	+	B.A. 16	+	B.A. 26	+
Cheese, 3 months	B.A. 7	+	B.A. 17	+	B.A. 27	+
Cheese, 6 months	B.A. 8	+*	B.A. 18	+*	B.A. 28	+†
Cheese, 1 year	B.A. 9	−	B.A. 19	−	B.A. 29	−

* Guinea-pig lesions slight. Cultures positive.
† Five guinea pigs negative. Three showed slight lesions. Cultures positive.

In series B.A. 11–19 results were identical with those obtained with series B.A. 1–9.

In series B.A. 21–29 the organism was fully viable at three months (table 1). At six months, five guinea pigs were negative. The three others were positive, but showed slight lesions. No infection was present in any of the guinea pigs inoculated with the sample one year old.

Experiment No. 4. Survival of Brucella abortus in Cheddar Cheese Manufactured from Milk from Selected Cows in a Highly Infected Herd, That Were Found to Be Eliminating Brucella abortus in the Milk

The cheese for this experiment was made up and handled in the same manner as that used in experiment 3 except that the milk was from a different reacting herd, and the milk was not fortified by the additional *Brucella abortus*. The milk contained 700 to 800 viable *Brucella abortus* per ml.

Inasmuch as the milk was only from two animals found to be eliminating the organism in the milk in large numbers, there was no dilution of the organism as occurs in pooled samples of milk from a given herd or area. We may therefore assume that the concentration of organisms was considerably greater than would occur under commercial conditions.

In series B.A. 31–39 the organisms were viable in the raw milk, fresh curd, and the stored cheeses up to and including one month, as shown in table 2. At three months all eight guinea pigs were positive, but the lesions were slight. At six months, five guinea pigs were negative, and three positive. In the three positive animals the lesions were slight. All guinea pigs inoculated with the one-year-old sample were negative.

In series B.A. 41–49 all samples were strongly positive up to and including the one-month cheese sample (table 2). At three months, seven guinea pigs were negative; one showed slight lesions but gave a positive culture. All guinea pigs inoculated with the six-month and one-year samples were negative.

TABLE 2

Survival of Brucella abortus in Cheddar cheese manufactured from milk from two selected cows in a highly infected herd, that were found to be eliminating Brucella abortus in the milk (Exper. No. 4)

Product and age	First series		Second series		Third series	
	Sample	Results	Sample	Results	Sample	Results
Raw milk	B.A. 31	+	B.A. 41	+	B.A. 51	+
Fresh whey	B.A. 32	+	B.A. 42	+	B.A. 52	+
Fresh curd	B.A. 33	+	B.A. 43	+	B.A. 53	+
Cheese, 1 week	B.A. 34	+	B.A. 44	+	B.A. 54	+
Cheese, 2 weeks	B.A. 35	+	B.A. 45	+	B.A. 55	+
Cheese, 1 month	B.A. 36	+	B.A. 46	+	B.A. 56	+
Cheese, 3 months	B.A. 37	+*	B.A. 47	+−‡	B.A. 57	+*
Cheese, 6 months	B.A. 38	+−†	B.A. 48	−	B.A. 58	+*
Cheese, 1 year	B.A. 39	−	B.A. 49	−	B.A. 59	−

* Guinea-pig lesions slight. Cultures positive.
† Five pigs negative. Slight lesions in three. Cultures positive.
‡ Seven guinea pigs negative. Slight lesions in one. Cultures positive.

In series B.A. 51–59 all samples were strongly positive up to and including the one-month sample. At three months and six months, all guinea pigs were positive, but the lesions were slight. All guinea pigs injected with the one-year sample were negative, as shown in table 2.

Experiment No. 5. Brucella abortus in Milk and Cheddar Cheese from Commercial Cheese Factories in Areas Where Brucellosis Had Not Been Eradicated from Producing Herds

Commercial cheese-milk samples, and fresh cheese curd were obtained for test for the presence of *Brucella abortus* in areas where little or no

attempt had been made to eradicate brucellosis from the herds. Milk and fresh curd were obtained from 22 different vats in 18 separate Wisconsin factories, and from 12 different vats in 9 New York factories. A smaller amount of cheese was inoculated into each guinea pig because of the fact that the pigs could not tolerate the larger amount used in the previous experiments. The probable explanation is that the milk used in experiments No. 3 and 4, which had bacterial counts below 10,000, was of a higher sanitary grade than that customarily used in commercial cheese manufacture.

All cheeses were held at the factory until the results of the inoculations for the presence of *Brucella abortus* were obtained. Cheeses made from positive milk were then purchased and brought to the laboratory, stored at 1.7° to 4.4° C., and examined further for the presence of *Brucella abortus*. It was our plan to obtain the cheeses promptly to test them at 60 days of age, but because of misunderstanding and food rationing, receipt was delayed in four instances.

Of the 22 lots of milk from the Wisconsin factories, five were positive for *Brucella abortus*. The remainder were negative as shown in table 3. Of the five positive samples, the cheeses could not be obtained in two instances. The cheeses had been released by mistake and could not be traced. The cheeses made from the other three samples were obtained and subjected to further testing for *Brucella abortus*. One cheese was negative for *Brucella abortus* on the first test at 66 days and later on second test at 144 days; the other two cheeses were negative at 84 and 115 days after manufacture. It is not known whether the cheeses were negative at earlier dates.

Of the 12 milk and fresh curd samples from the New York factories, eight milk samples were negative and four were positive (table 3). One sample of positive milk gave fresh cheese curd that was negative. All four samples of cheese from the positive milk were negative in first test. Two of these four were negative for *Brucella abortus* at 74 days and 104 days. The other two were found to be negative at 41 days and also at 65 days. Again, it is not known just how soon after manufacture these cheeses actually became negative as they were negative in first test.

DISCUSSION

A very serious obstacle in the study of the survival of most pathogenic bacteria in commercial cheese is the difficulty of obtaining vats of cheese milk known to be infected with the bacteria under observation which would normally be made into cheese. It is generally impossible to determine the extent of the contamination that naturally occurs in the milk or to follow the survival of the bacteria in the cheese made under commercial conditions as no one can predict in advance that a given vat of milk is infected with specific disease-producing bacteria. Under such circumstances it has been the accepted research practice to artificially fortify milk with selected patho-

TABLE 3

Brucella abortus in milk and Cheddar cheese from commercial cheese factories in areas where brucellosis had not been eradicated from the herds (Exper. No. 5)

No.	Factory	Milk	Curd	Cheese†	No.	Factory	Milk	Curd	Cheese†
1	Wis. G.V.	–	–	.....	18	Wis. J.	–	–	.....
2	Wis. W. 1	–	–	.....	19	Wis. Sp.	–	–	.....
3	Wis. W. 2	–	–	.....	20	Wis. Do.	–	–	.....
4	Wis. B.A.	+*	+*	Negative at 66 days Negative at 144 days	21	Wis. G.A.	+*	+*	Unobtainable
5	Wis. B.B.	–	–	.....	22	Wis. S.B.	+*	+*	Unobtainable
6	Wis. Bo. A.	–	–	.....	23	N.Y. C. 1	–	–	.....
7	Wis. Bo. B.	–	–	.....	24	N.Y. C. 2	+	+	Negative at 74 days Negative at 104 days
8	Wis. H.	–	–	.....	25	N.Y. C. 3	–	–	.....
9	Wis. C.	–	–	.....	26	N.Y. C. 4	+	+	Negative at 74 days Negative at 104 days
10	Wis. D.A.	–	–	.....	27	N.Y. L. 1	–	–	.....
11	Wis. D.B.	–	–	.....	28	N.Y. L. 2	+	+	Negative at 41 days Negative at 65 days
12	Wis. S.	–	–	.....	29	N.Y. L. 3	–	–	.....
13	Wis. H.C.	–	–	.....	30	N.Y. L. 4	+*	–	Negative at 41 days Negative at 65 days
14	Wis. Na.	–	–	.....	31	N.Y. L. 5	–	–	.....
15	Wis. G.M.	+*	+*	Negative at 84 days Negative at 115 days	32	N.Y. L. 6	–	–	.....
16	Wis. O.	+*	+*	Negative at 84 days Negative at 115 days	33	N.Y. L. 7	–	–	.....
17	Wis. C.L.	–	–	.....	34	N.Y. L. 8	–	–	.....

* Guinea pigs showed slight lesions. Cultures positive.

† The only tests made on the cheeses are those reported in the table.

gens and then manufacture this milk into cheese under laboratory conditions. All of the controlled experiments on the survival of *E. typhosa, Mycobacterium tuberculosis,* and *S. pyogenes* in cheese have been conducted in this manner. Such experiments yield valuable information but may not apply to commercial cheese as the size of the inoculation naturally occurring in milk is unknown and the actual longevity and virulence of the bacteria causing the contamination may differ from those cultured in the laboratory. The only other alternative is to study cheese that has been suspected of producing a cheese-borne epidemic to determine the probable age of the cheese and to attempt to isolate the bacteria involved.

Fortunately for research purposes, the survival of *Brucella abortus* in cheese may be studied more advantageously than other pathogens. Freshly isolated cultures were used to fortify milk for cheese making, but it was also possible to find naturally infected milk delivered to factories in areas where brucellosis had not been eradicated. Milks from many sections were tested to obtain supplies containing *Brucella abortus* which provided naturally infected milk that could be made into cheese. Hence, the survival of *Brucella abortus* could be studied in cheese made by accepted laboratory procedures and under practical conditions.

The data on Cheddar cheese manufactured in the laboratory from milk fortified with a culture of *Brucella abortus* or from milk produced by reacting cows whose milk was found to be highly contaminated with these bacteria showed that *Brucella abortus* survived in cheese at 4.4° C. for six months but not for one year. These bacteria tended to die a little more rapidly in cheese made from the milk selected as being naturally heavily contaminated. These data agree well with those recently published by Campbell and Gibbard (3) for *E. typhosa* in Cheddar cheese made from fortified milk, and with those of Yale and Marquardt (18) for *S. pyogenes.* The time of survival of *Brucella abortus* in Cheddar cheese is much longer than that obtained earlier by Wade and Shere (17) who manufactured Cheddar cheese from milk fortified with *E. typhosa* and found most of the bacteria dead in 36 days at 15.5° C.

The results obtained on commercial cheese milk and commercial cheese are especially interesting. In 1942, 15 vats of Cheddar-cheese milk were all negative. The 10 vats of Limburger-cheese milk contained two positives but the cheese curds from both positive milks were negative. In 1944, in the 34 vats of Cheddar cheese milk selected in areas in Wisconsin and New York where brucellosis had not been eradicated, there were nine positive for *Brucella abortus,* and the curd from one of the positive milks was negative. In the two surveys there were a total of 59 milk samples with 11 positives, but only eight positive cheese curds as taken from the vat. Of the cheeses made from positive milks nine were recovered for examination and all were negative on the first tests which were made after the cheeses had been stored

for 41 to 84 days at 1.1° to 2.7° C. Fortunately, the two cheeses made from the most heavily infected raw milks were tested after 41 and 74 days aging. It is obvious, therefore, that *Brucella abortus* died much more rapidly in commercial cheese than in the fortified laboratory samples.

The question naturally arises as to the reason for the much more rapid death of *Brucella abortus* in commercial cheese as compared with inoculated laboratory samples. The obvious answer is that the commercial cheese had a very much lower initial contamination. This is indicated by the commercial manufacture of three lots of negative cheese curd from positive vats of milk. The evidence indicates that *Brucella abortus* did not multiply rapidly in the cheese milk and curd during the manufacturing process. If it had, the fresh curd in which the bacteria were held and concentrated should have contained more bacteria than the gravity cream from the milk used for guinea-pig injection. It should be pointed out that the size of the inoculum was less for commercial than for laboratory cheeses but in each case the dose was the maximum the animal could take subcutaneously.

The data indicate that aging for about 60 days is reasonable assurance of the absence of viable *Brucella abortus* in commercial Cheddar cheese. Cheddar cheese has not been proved to have produced undulant fever and most of this cheese under normal conditions has been consumed after two months of age. These results may not apply to the survival of *E. typhosa*. It may never be possible to definitely prove the time of survival of *E. typhosa* in commercial cheese except by the data on the limited numbers of cheese-borne typhoid epidemics. The evidence cannot be based upon cheese made from milk fortified with the pathogenic bacteria for such experiments would warrant the false conclusion that raw milk Cheddar cheese should be aged for one year to assure absence of *Brucella abortus* and *E. typhosa*. Except for the statement of Wade and Shere (17) that *E. typhosa* did survive in commercial Cheddar cheese for 63 days, the history of cheese-borne typhoid epidemics shows that no cheeses over 60 days of age were involved. The epidemiological evidence of Thomasson (13) is clear that *E. typhosa* survived in Colby type cheese for 45 days. Campbell and Gibbard (3) review the work of Bowman (1) and state that "evidence was presented indicating that the cheese remained dangerous for at least 80 days." Now Bowman (1) very carefully reported his findings on the typhoid fever epidemic due to cheese and he made no statement on the longevity of *E. typhosa* in Cheddar cheese for the data were not exact on this point. If the 80 days survival is to be accepted as proved, then one must know the identity of each cheese in the hands of a wholesaler who, Bowman stated, "kept no records," and the statement of Bowman that "we suspect that it was two cheeses from this vat No. 108" must be accepted as an established fact. The conclusions of Bowman are that cheese did cause the typhoid fever epidemic and that "either pasteurization of milk or adequate aging of cheese should be insisted

upon." Bowman must have believed the evidence was not adequate to give data on the actual maximum survival of *E. typhosa* in Cheddar cheese for it was not mentioned by him.

The situation is well stated by Brooks (2) in his 1944 report of the committee on communicable diseases affecting man which was devoted to "cheese-borne infection" when he stated, "If we assume that such terms as 'short-cured,' 'fresh,' 'green' and 'unripened' indicate storage for less than 60 days, a fairly safe assumption, then none of the outbreaks cited have been traced to cheese cured for this length of time." The experience of the epidemiologist with cheese-borne typhoid fever epidemics substantiates the data in this study on the survival of *Brucella abortus* in commercial Cheddar cheese that about 60 days aging is reasonable assurance of the absence of these viable pathogenic bacteria. An exception is *Brucella melitensis* in goat's milk cheese, as shown by Stiles (12), and goat's milk cheese presents a special problem.

CONCLUSIONS

Cheddar cheese was made from milk produced by a herd highly infected with brucellosis. The milk contained about 500 viable *Brucella abortus* bacteria per ml. and was then inoculated with a freshly isolated culture to contain about 1000 organisms per ml. *Brucella abortus* survived in the cheese for six months at 4.4° C. but the cheeses were all negative in one year as determined by guinea-pig inoculations.

When the milk was produced by reacting cows selected for eliminating large numbers of *Brucella abortus* the plate count was 700 to 800 *Brucella abortus* per ml. This pathogen remained viable in all the Cheddar cheeses made from such milk for three months, it could not be demonstrated in some cheeses in six months, and it was negative in all tests on guinea pigs in one year.

Tests were conducted on 10 vats of commercial cheese milk made into Limburger cheese and 15 vats made into Cheddar cheese produced in New York areas selected at random. There were two vats of Limburger-cheese milk positive for *Brucella abortus* with guinea pig inoculations and no Cheddar-cheese milk was positive. The Limburger curds made from the positive milks were negative and the cheese was negative on first test after 57 days storage.

Tests were also made on 34 vats of commercial Cheddar-cheese milk produced in areas in New York and Wisconsin where brucellosis had not been eradicated from the milking herds. There were nine positive milks and eight positive curds. Two cheeses were lost. The other seven cheeses made from positive milks were all free from viable *Brucella abortus* bacteria on the first examination as determined by guinea-pig inoculations. These tests were made after storage of the cheeses for 41 to 84 days at 1.1° to 2.7° C.

The data on all commercial cheeses gave a total of 59 vats of commercial cheese milk of which 11 were positive for *Brucella abortus*. There were three vats of positive milk that gave negative fresh curds. Two cheeses made from positive milks could not be located. The Cheddar cheeses from the nine positive milks were all negative on first examination after storage at 1.1° to 2.7° C. for periods varying from 41 to 84 days. No *Brucella abortus* organisms were recovered from any sample of commercial cheese.

It was pointed out that Cheddar cheese has not been proved to be a carrier for undulant fever and that reported typhoid fever epidemics have not been attributed to cheese cured for more than 63 days. These observations in the literature are of interest in considering the experimental data on commercial cheese in this study which show that an aging period of 60 days is reasonable assurance against the presence of viable *Brucella abortus* organisms in Cheddar cheese.

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THE CALCULATION OF TOTAL SOLIDS BY MEANS OF THE SHARP AND HART EQUATION

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In 1936, Sharp and Hart (4) published a careful study of the factors influencing the estimation of total milk solids in milk from measurements of specific gravity and fat content. As a result of their studies, they derived the equations:

$$\text{Total solids} = 1.2648\ \text{Fat} + 0.2586\ \text{Lactometer} \qquad \text{I}$$

and

$$\text{Total solids} = 1.2537\ \text{Fat} + 0.2680\ \frac{\text{Lactometer}}{\text{Sp. gr. milk}} \qquad \text{II}$$

The first equation is theoretically less accurate but more convenient to use. Since that time, several reports have reached the writer that the values calculated by Sharp and Hart's equation were too low. For example, a mimeographed "Report of Committee for Milk and Cream, American Dairy Science Association—June, 1943," contains the following statement:

> In the case of the Sharp procedure all laboratories found it to give results considerably lower than those obtained with the Mojonnier test. The average deviation ranging from −0.53 to −0.76%.

It is the purpose of this paper to point out one source of this error.

Sharp and Hart did not use a lactometer for their measurements. Their data were obtained by means of a modified Westphal balance and they recorded the specific gravity of milk as the ratio of the weights of equal volumes of milk and water when both were *at 30° C.*

They made an error when they attempted to convert these values to readings on a Quevenne lactometer. They overlooked the fact that their specific gravities were based upon water at 30° C. while the Quevenne lactometer gives specific gravities based upon water at 60° F. Values of specific gravity at 30° C./30° C. must be multiplied by 0.99663 to convert them to values at 30° C./60° F.

When a Quevenne lactometer is used at 30° C. it does not indicate exactly the specific gravity referred to water at 60° F., because there is an appreciable expansion of the lactometer when it is warmed from 60° F. to 30° C. If we assume that the value 0.00002648 given for the cubical coefficient of expansion of glass in the Handbook of Chemistry and Physics (1) is applicable to the ordinary lactometer, then we can convert true values of specific gravity at 30° C./60° F. to apparent specific gravities as measured with a lactometer, by multiplying by 1.00038. By multiplying these two correc-

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tion factors together, we can combine them into a single factor: 0.99701. Hence, the specific gravity data of Sharp and Hart can be converted to equivalent Quevenne readings at 30° C. by the formula

$$Q. = 1000(0.99701 \times \text{Sp. gr.} - 1.0000) \qquad \text{III}$$

from which

$$\text{Sp. gr. } 30°/30° = \frac{Q. + 1000}{997} \qquad \text{IV}$$

The Sharp and Hart equations may be rewritten in terms of the specific gravities at 30°/30° from which they were derived.

$$\text{Total solids} = 1.2648\ \text{Fat} + 258.6\ (\text{Sp. gr.} - 1.0000) \qquad \text{V}$$

and

$$\text{Total solids} = 1.2537\ \text{Fat} + 268.0\left(\frac{\text{Sp. gr.} - 1.0000}{\text{Sp. gr.}}\right) \qquad \text{VI}$$

Substituting the value of Sp. gr. (Equation 4) in the second term of equation 5 gives $\frac{258.6}{997}(Q. + 1000 - 997)$ which reduces to $0.2594\ (Q. + 3)$. The complete corrected equation becomes

$$\text{Total solids} = 1.2648\ \text{Fat} + 0.2594\ (Q. + 3) \qquad \text{VII}$$

In the case of equation VI two substitutions are necessary, because the Sp. gr. at 30°/30° appears twice in the equation. These substitutions yield the following corrected equation:

$$\text{Total solids} = 1.2537\ \text{Fat} + \frac{268.0\ (Q. + 3)}{Q. + 1000}$$

If Quevenne lactometer readings are substituted directly into the original Sharp and Hart equation, the results for total solids will be about three-fourths of a per cent lower. This is approximately the error which has been observed by some users of the equation.

After the preceding paragraphs were written, two papers were found which deal with this same problem. Rueda (3) recognized that a correction must be applied to the ordinary lactometer readings before they could be used in the Sharp and Hart equation. He attempted to determine the magnitude of the correction by comparing the actual reading of a lactometer in a sucrose solution at 30° C. with the actual specific gravity at 30°/30° as determined by a pycnometer. In this way, he obtained a correction of 3.2° C. This differs by 0.2° Q. from the value calculated by the writer.

It is not clear from Rueda's paper whether he had checked the accuracy of his lactometer at 60° F. If not, then the small difference of 0.2° Q. might be due to a minor error in calibration of the lactometer. There are other possibilities. A hydrometer is affected by surface tension as well as by density. A liquid of higher surface tension will appear to have a lower density than another of identical density but lower surface tension. The difference of 0.2 might be due to differences in the surface tension of the

sugar solution and of the solution used to check the accuracy of calibration of the lactometer. The reading of an ordinary Quevenne lactometer in pure water can be raised approximately four degrees by touching the surface of the water with a piece of soap.

Livak (2) calculated a correction based on the difference between the densities of water at 30° C. and at 60° F. Because he overlooked the effect of temperature upon the lactometer itself, he obtained a value of 3.5° Q. Livak went one step further and compared total solids calculated by a corrected Sharp and Hart equation with the results of Mojonnier analyses. These comparisons indicated that his correction of 3.5° and Rueda's of 3.2° were both too large. In order to make the calculated values agree with Mr. Livak's gravimetric values, it would be necessary to use a correction of approximately 2.3 in the Sharp and Hart equation.

It is not the purpose of the present writer to question or to defend the experimental work of either Livak or of Sharp and Hart which led to a difference of 0.7° Q. in the value of the correction. It is his intention only to point out the difference between actual lactometer readings and the calculated values used by Sharp and Hart, and to calculate the magnitude of this difference.

SUMMARY

There is an error in the derivation of the Sharp and Hart equations. By correcting this error, an equation is obtained which yields values for total solids which are approximately three-fourths per cent higher.

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THE VITAMIN A AND CAROTENE CONTENT OF MARKET BUTTER PRODUCED IN KANSAS[1]

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Great interest has arisen in the nutritional value of butter as a result of problems arising from the wartime food situation. In 1941 the Committee on Food and Nutrition of the National Research Council suggested to the Secretary of Agriculture that a study of the vitamin A potency of creamery butter should be undertaken. Through the cooperation of the Bureau of Dairy Industry and the Office of Experiment Stations a project was developed in which 20 experiment stations have participated in making a survey of the vitamin A potency of creamery butter produced in this country. This paper presents the results on butter produced in Kansas during the period July, 1943, through December, 1944. In addition to the determination of vitamin A and carotene, certain other data were obtained on butter composition and quality, pasture conditions and feeding practices, in an attempt to determine whether these factors are related to the vitamin A and carotene content of butter.

A review of the literature on this subject will be unnecessary since an excellent list of references relating to this problem has been published (7).

EXPERIMENTAL

Sampling. Butter was obtained from seven creameries located in different areas of the state (fig. 1). Six of these creameries, four of which are cooperatives, obtain cream from local sources. The seventh, Creamery F, is a large centralizer using cream from widely scattered sources. These seven creameries made about 20 per cent of the butter produced in Kansas during 1944. The samples were collected during the latter half of each month and represented small aliquots of each churning for the day samples were obtained. These samples, packed in small, tightly closed glass jars, were sent to the laboratory without refrigeration during transit. Except for a few samples sent during the hottest part of the summer, they arrived in a firm condition. Upon arrival the samples were placed in a refrigerator until analysis could be made. Probably no loss of vitamin occurred in handling butter in this manner since it has been shown that butter held for long periods under refrigeration or for even as much as three days at room temperature lost no vitamin A (2, 7). The samples were softened and mixed thoroughly before sampling for analysis.

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[1] Contribution No. 302, Department of Chemistry, and No. 161, Department of Dairy Husbandry.

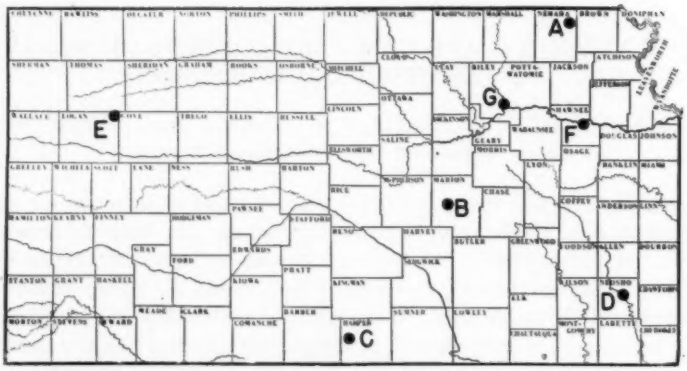

FIG. 1. Location of the seven cooperating creameries.

Methods of Analysis and Collection of Supplementary Data. Vitamin A and carotene were determined by methods developed under the direction of the Technical Committee on Vitamin A Researches (8, 9) with the following minor changes. A Coleman spectrophotometer, model 11, set at 620 mμ, was used for determining vitamin A by the Carr-Price reaction. During the course of this study it was discovered that the stability of the blue color was increased and the ease of reading the galvanometer was improved by decreasing the light intensity of the exciter lamp of the photometer through the use of a resistance unit (3). Carotene and total color (as carotene) were both read at 460 mμ. The standard calibration curves were essentially straight lines having the slopes shown by the K values in table 1.

The factor 0.005 was found to correct for the blue color produced by the reaction of antimony trichloride and carotenoids in concentrations of 2–10 micrograms per milliliter. This is similar to the concentrations of pigment encountered in the butter samples. Unlike the results reported by Jenness and Palmer (7), the slope of the calibration curve of the β-carotene and

TABLE 1

Calibration of the Coleman spectrophotometer for vitamin A analysis

Substance	Solvent	Range of concentration	Wave length	K*
		μg./ml.	mμ	
Vitamin A alcohol (Dist. Prod. Co.)	Chloroform	1.0–6.0	620	14.6
β-Carotene (S.M.A.)	"	0.2–2.5	460	3.35
β-Carotene (S.M.A.)	Skellysolve B	0.2–2.5	460	3.0

* $K = \frac{\mu g./ml.}{2 - \log G}$ (for vitamin A); $K = \frac{\mu g./ml.}{\text{optical density}}$ (for β-carotene). G is the galvanometer reading with the galvanometer previously set at 100 on a mixture of 9 ml. of antimony trichloride reagent and 1 ml. of chloroform.

antimony trichloride reaction-mixture was found to increase rapidly at higher concentrations; however, fairly satisfactory results were obtained for the concentrations used in this study. Methods for making calibration curves and determining the correction factor have been described elsewhere (4, 9, 10).

The color of the butter was compared to the Nafis standard, and it was scored for quality in the usual manner.

Data on butter production were obtained from the Dairy Commissioner of Kansas and the reports of the Bureau of Agricultural Economics of the United States Department of Agriculture. Pasture conditions, feeding practices, etc., were supplied by county agents of the areas in which the creameries were located.

RESULTS

Content and Variation in Vitamin A Potency of Kansas Butter. The values for the vitamin A and carotene content of butter included in this study are shown in table 2. The values are weighted for the monthly production of each of the cooperating creameries. The factors used to convert the values to International Units will be discussed in another section of this report. The correction made for vitamin A lost in analysis is based on a recovery of 93 per cent when vitamin A was added to butter before analysis.[2]

The mean vitamin A potency for the 1944 "winter" butter was 11,050 I.U. per pound, compared to 17,700 I.U. per pound for the "summer" months, and 15,100 I.U. per pound for the mean annual value. The values for corresponding months of 1943 and 1944 (table 2) show the vitamin A potency was appreciably higher in 1944 for the months of September, October, and November. In the fall of 1943 the grass was not as good as in the fall of 1944 when plentiful rainfall and mild weather produced particularly good pasture. In some parts of the state abundant cereal grass pasture was available until late in the year.

The mean annual vitamin A potency is slightly higher than the value reported by Jenness and Palmer from Minnesota (7), but the difference is unimportant since they apparently made no correction for vitamin A lost in analysis.

The vitamin A potency of Kansas butter was lowest in January and February (values of 9,350 and 8,950 I.U. per pound respectively), but increased rapidly from April to June, reaching a high of 20,000 I.U. per pound. Through July and August the values dropped off somewhat but again increased in the fall. The values for both vitamin A and carotene follow the same general trend, although some variation in the relative positions occur from month to month, and the change for vitamin A was less than the change for carotene. The mean carotene content increased from 1.63 micrograms per gram in February to 5.25 micrograms per gram in June,

[2] C. A. Cary. Unpublished communication.

the latter value being 3.2 times higher. Even slightly greater values were obtained in November (table 2). In the same months the vitamin A values were 3.95 and 8.2 micrograms per gram, or 2.1 times higher in June. In Minnesota during 1943, almost a fourfold change was found in the carotene content of the butter from the fourth to the sixth 4-week sampling periods.

TABLE 2

The vitamin A potency of Kansas market butter[a]

Year and month of production	Distribution of annual butter production	Carotene per gram	Vitamin A per gram	Potency per pound[b]		Vitamin A potency due to carotene
				Not corrected for loss in analysis	Corrected for loss[c] of vitamin A in analysis	
	% per mo.	*μg.*	*μg.*	*I.U.*	*I.U.*	*% of total*
1943:						
July	10.13	5.61	5.92	15,000	15,800	26.8
August	8.55	5.32	4.88	12,900	13,550	29.6
September	6.82	3.62	6.33	14,200	15,100	18.2
October	5.94	3.53	5.44	12,550	13,300	20.0
November	6.15	3.28	4.94	11,500	12,100	20.4
December	6.04	2.42	4.69	10,350	11,000	16.6
1944:						
January	7.25	1.86	4.07	8,800	9,350	15.0
February	8.21	1.63	3.95	8,400	8,950	13.8
March	9.11	1.74	5.15	10,700	11,400	11.5
April	9.16	2.65	6.07	13,050	13,850	14.5
May	11.63	5.02	8.15	18,600	19,750	19.3
June	11.16	5.25	8.2	18,850	20,000	19.9
July	10.18	5.03	6.19	15,050	15,950	23.9
August	8.82	4.71	6.25	14,900	15,800	22.6
September	7.13	4.70	6.67	15,700	16,600	21.5
October	5.83	4.88	6.89	16,200	17,150	21.6
November	6.02	5.51	6.69	16,300	17,250	24.2
December	5.73	3.32	4.44	10,600	11,300	22.4
Mean, July, 1943, to June, 1944[d]				13,370	14,170	19.8
Mean, December to April, 1944[d, e]				10,400	11,050	14.9
Mean, May to November, 1944[d, f]				16,800	17,700	21.4
Mean, annual, 1944[d]				14,250	15,100	19.6

[a] All values represent the mean, weighted according to production for the seven creameries sampled.

[b] 0.6 microgram carotene = 1 I.U.; 1 microgram of vitamin A = 4 I.U.

[c] Corrected for loss in analysis, based on recovery of 93 per cent of vitamin A.

[d] Weighted for monthly production.

[e] Production for December to April, inclusive, was 39.2 per cent of the annual output.

[f] Production for May to November, inclusive, was 60.8 per cent of the annual output.

The low and high vitamin A values were observed in the third and ninth periods, the value being about 1.9 times higher during the latter period (7).

The trends in the vitamin A potency can be seen to follow the seasonal and pasture conditions. In January, February, and often into March little pasture is available over most of the state. But with the approach of spring both cereal and native grass pastures develop rapidly. The carotene and vitamin A values of the butter clearly reflect this change in feeding condi-

tions. The drop in the values during July and August is probably due to the effect of hot weather. In areas of low rainfall the pastures rapidly deteriorate during hot weather. However, the possibility that a part of the decrease in the vitamin content may be due to the effects of weather on the milk, cream, or butter cannot be overlooked.

The values shown for 1944 follow a trend similar to those found by Jenness and Palmer for corresponding months of 1943 (7), although Kansas

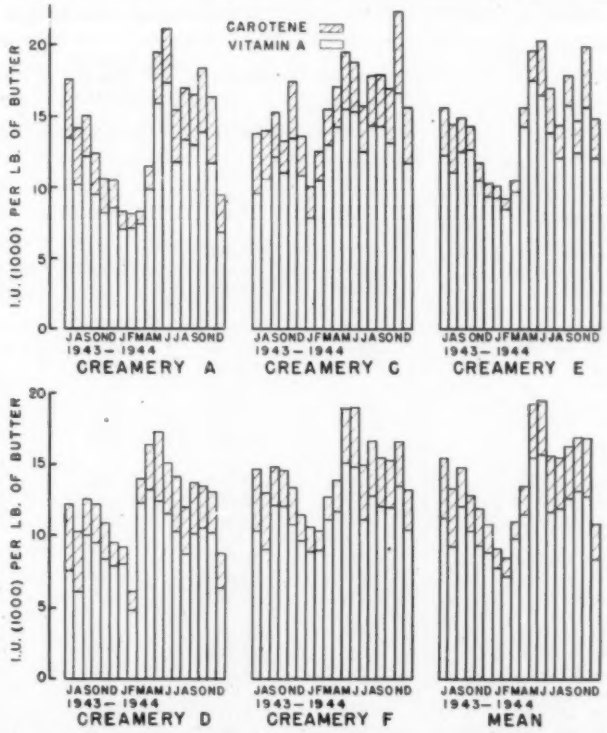

FIG. 2. Monthly variations in vitamin A potency due to carotene and vitamin A. Letters beneath the bars indicate the months. The mean represents values which have been weighted for the production of each of the seven creameries included in this study.

values show appreciable increases in March and April which are not shown in Minnesota. While the Minnesota values in the fall were fully as high as in the spring, there were considerable decreases during the last two 4-week sampling periods of the year. The values obtained in this study were high for September, October, and November but dropped off sharply in December. The geographical locations of the two states suggest an explanation for these results. No doubt milder winters, lasting for a shorter length of time,

makes pasture available in Kansas for a greater part of the year. The cereal grasses are generally available for pasturage early in the spring and late in the fall, providing an excellent source of carotene in addition to the native grasses and dry roughage. In fact, in many parts of the state when the weather is mild, occasional use may be made of wheat pasture during the winter months.

TABLE 3

Mean vitamin A potency of butter produced by seven Kansas creameries in 1944

	Carotene	Vitamin A*	Potency due to carotene	Total vitamin A potency*
	I.U./lb.	*I.U./lb.*	*% of total*	*I.U./lb.*
Creamery A				
Mean, Dec. to Apr.	1,500	8,050	15.7	9,560
Mean, May to Nov.	3,900	14,400	21.2	18,400
Mean, annual	2,900	11,800	19.7	14,700
Creamery B				
Mean, Dec. to Apr.	1,200	8,600	12.3	9,800
Mean, May to Nov.	3,700	14,400	20.2	18,200
Mean, annual	2,670	12,000	18.2	14,700
Creamery C				
Mean, Dec. to Apr.	2,730	12,050	18.5	14,800
Mean, May to Nov.	3,940	15,000	20.8	18,950
Mean, annual	3,460	13,850	20.0	17,300
Creamery D				
Mean, Dec. to Apr.	2,020	9,750	17.2	11,800
Mean, May to Nov.	3,750	11,000	25.4	14,700
Mean, annual	3,140	10,550	23.0	13,700
Creamery E				
Mean, Dec. to Apr.	1,140	11,000	9.4	12,200
Mean, May to Nov.	2,970	15,600	16.0	18,550
Mean, annual	2,170	13,550	13.8	15,700
Creamery F				
Mean, Dec. to Apr.	1,910	10,650	15.2	12,600
Mean, May to Nov.	3,740	13,600	21.6	17,350
Mean, annual	3,020	12,450	19.5	15,500
Creamery G				
Mean, Dec. to Apr.	1,450	9,250	13.6	10,700
Mean, May to Nov.	4,020	14,700	21.4	18,700
Mean, annual	2,790	11,900	18.5	14,600
Mean of seven creameries†				
Mean, Dec. to Apr.	1,640	9,400	14.9	11,050
Mean, May to Nov.	3,800	13,900	21.4	17,700
Mean, annual	2,950	12,100	19.6	15,100

* Corrected for loss in analysis, based on recovery of 93 per cent vitamin A.
† Weighted for production.

The variations in the vitamin A, carotene, and total vitamin A potency of butter produced in the different areas is shown in table 3. Butter from creamery C, located in the southern part of the state where considerable use is made of cereal grass pasture, had the highest "winter" as well as the highest annual vitamin A potency. The "summer" mean is not appreciably higher than that of the butter from several other creameries. This creamery also makes a butter of considerably higher carotene content in the winter

months than was found for creamery A in the northeastern or creamery E in the northwestern parts of the state (fig. 2). But the differences in the carotene-content of the butters from these three creameries tend to vary much less in the summer and fall months. The availability of pasture in the different areas is probably the explanation for these results.

Creamery D, in the southeastern area, produced butter of the lowest annual mean vitamin A potency. It is interesting to note that butter from this source contains a higher per cent of the total vitamin A potency as carotene than does butter from any other creamery except creamery C during the "winter" period (table 3). A greater percentage of cows of the Jersey breed are found in this area than in any of the others included in this study.

During the winter the percentage of the total vitamin A potency due to carotene is less than during the summer months. The mean value is 14.9 per cent in the "winter" and 21.4 per cent in the "summer" months. Carotene accounted for 6.5 per cent of the vitamin A potency of the March butter from creamery E and 27.8 per cent of the vitamin A potency of the November butter made by creamery A. These values represent the extreme variations of potency due to carotene as found in this study.

An attempt was made to correlate the Nafis color grade with the vitamin content of the butter. While there was a tendency for the colors to be a little lighter in the winter months, the use of artificial colors made impossible a meaningful correlation study. All except two of the samples of butter graded either A or AA by the Nafis color standards.

No correlation was found between butter score and vitamin A potency. These findings agree with those previously reported (2).

DISCUSSION

Notes on the Factors for Converting the Vitamin A and Carotene to Units of Potency and on Nutritional Significance. The potency of 0.6 micrograms of β-carotene has been adopted as 1 I.U. (6). However, there is still considerable question about the conversion factor for vitamin A. Baxter and Robeson found that 1 microgram of vitamin A averages 4.3 U.S.P. Units (1). Although U.S.P. Units and International Units were originally set as equivalent to each other, there is evidence of variability and loss of potency in the U.S.P. Reference Oil[3] (11). Gridgeman recently has summarized the work of a number of investigators which deals with these conversion factors (5). The use of 1 microgram of vitamin A as equivalent to 4 I.U. has been adopted for the results reported in this paper. This is probably the most satisfactory factor available at the present time. Since this factor already has been adopted by other laboratories participating in this study of butter (2, 7), the results of the different investigations may be compared.

The rat has been used as the test animal for establishing the relationship

[3] H. G. Wiseman. Personal communication.

of β-carotene and vitamin A to units of potency. The factor which will represent the conversion of these substances to units of vitamin A potency by humans has not been investigated sufficiently to warrant the use of a value more refined than that found in the rat-feeding investigations.

The material determined as β-carotene by the methods employed in this investigation may include other carotenoids as well as optical isomers of β-carotene. Variations in these constituents may cause the true vitamin A potency of butter to differ by a few per cent from the values found by analysis.

The limitations of the factors for converting vitamin A and carotene to units of vitamin A potency should be realized, especially when interpreting these results in terms of the requirements for human nutrition.

The results of the study of the vitamin A potency of butter reported to date indicate butter is probably a richer source of vitamin A than has been shown by many of the older nutrition tables which frequently give average values of 9,000–10,000 I.U. per pound. This study indicates Kansas butter has a mean potency possibly 50 per cent higher than this value.

It should be noted that in the late spring and early summer, a period of high vitamin A potency, more butter is produced than in the winter months when the vitamin A potency is lowest (table 2). Normally considerable quantities of butter go into storage during months of high production and will be placed on the market when the demand is greater. No loss of vitamin A potency has been found to occur when butter is stored under refrigeration (7), and the consumer may obtain butter of high vitamin A potency during periods when the butter being produced is of lower vitamin content. Since this condition exists, the mean annual vitamin A potency of butter probably represents the best indication of what the consumer obtains when buying butter for dietary requirements.

SUMMARY

1. The 1944 mean annual vitamin A potency of Kansas butter was 15,100 I.U. per pound. The mean for the period December to April was 11,050 I.U. per pound, and from May to November, 17,700 I.U. per pound.

2. In the above periods carotene accounted for 19.6 per cent, 14.9 per cent, and 21.4 per cent of the total vitamin A potency of Kansas butter.

3. The vitamin A and carotene content of butter produced in different areas of the state and during different months of the year varied with the pasture available. When little or no pasture existed the values for vitamin A and carotene dropped. Good pasture conditions increased the vitamin A and carotene values to maximum in May and June. During the hot summer weather the values dropped off somewhat, returning to higher levels when pastures improved in the fall of the year.

4. No correlation was found between the Nafis color grade or butter score and the vitamin A potency of butter.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the creameries supplying the butter samples for this study and to Professor W. J. Caulfield who assisted with the collection and scoring of part of the samples.

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SOME OBSERVATIONS ON THE ACID INVERSION OF SUCROSE

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When sugar rationing went into effect early in 1942, the dairy manufacturing industry, particularly ice cream manufacturers, became concerned about supplies of sweetening materials. Among other ingredients which evoked the interest of ice cream manufacturers, invert sugar sirup received considerable attention. During the ensuing two-year period there appeared in technical and semi-technical literature a great deal of controversial discussion concerning the relative sweetness of invert sugar sirup. Very few of these discussions were based upon experimental data.

Of all the probable factors related to sweetness of invert sirup, apparently no consideration has been given to variations in the composition of the sirup resulting when the inversion process is not carefully controlled. It has been tacitly assumed that the sucrose is always completely hydrolyzed during inversion, and that the resulting "invert" sugar is always composed of exactly equal quantities of dextrose and levulose (as theoretically it should be).

It was thought that a systematic study of some of the factors affecting the inversion of sucrose might aid in resolving some of the confusion concerning the sweetness of invert sugar. No organoleptic sweetness comparisons were made in this investigation, the primary object being to obtain some basic information on the applied chemistry of sucrose inversion. Furthermore, it was hoped that these experiments might reveal procedures for making invert sirup that would be more widely applicable than methods which have been recommended heretofore.

REVIEW OF LITERATURE

Very little detailed information is available relative to the effect on the inversion of sucrose of such factors as kind of acid used, pH, temperature employed, and time of holding at the hydrolyzing temperature. These are all conditions which may, either intentionally or inadvertently, be varied from one batch to another.

Taussig (6, 7) recommended boiling a solution of sugar and citric acid to a temperature of 232.5° F., followed by sudden cooling. He stated also that satisfactory results could be obtained with a temperature of 210°–218° F. maintained for an hour, followed by raising the temperature to the point "just below caramelization" then sudden cooling.

Baudrexel (2) reported boiling for 15 to 30 minutes a solution consisting of 100 kg. sucrose, 27–30 kg. water, 0.5 kg. $CaCl_2$, 1 kg. NaCl, and 300 g. citric acid (or 150–250 g. tartaric acid).

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In 1929 Schaal and Johnson (5) reported that beet sugar required more acid than cane sugar for satisfactory inversion because of the greater buffering capacity of the beet sugar. Their recommended procedure was to boil the sugar-water solution for 30 minutes then cool immediately. They found that for the above procedure the optimum pH was about 2.5. These workers apparently considered that approximately 90 per cent inversion was satisfactory in the production of commercial invert sirup.

EXPERIMENTAL PROCEDURE

The investigation reported in this paper was divided into four phases, as follows: *A*—Effect of kind of acid and pH on sucrose inversion. *B*—Effect of concentration of solution on sucrose inversion. *C*—Effect of boiling time on sucrose inversion. *D*—Time, temperature, and pH as factors affecting sucrose inversion.

Cane sugar was employed as the source of sucrose in the experiments reported herein.

A. Effect of kind of acid and pH on sucrose inversion. The acids used in this portion of the study were tartaric, citric, lactic, acetic, hydrochloric, sulfuric, and phosphoric. From each acid was prepared a solution of suitable concentration for obtaining the desired pH range when the acid solution was added to the sirup in varying quantities. The following amounts of acid in 100 ml. of solution were found to be satisfactory: tartaric, 30 grams; citric, 20 grams; lactic, undiluted; acetic (glacial), undiluted; hydrochloric, 3 ml. concentrated; sulfuric, 2 ml. concentrated; phosphoric, 3 ml. of 85%. The amounts of these solutions used are indicated in the data tables given under "Results."

All sirups were prepared by mixing together 1 lb. sucrose, 0.44 lb. water, and the required amount of acid solution. These solutions were heated quickly to boiling in a 1-liter beaker by placing on a wire gauze over a Bunsen burner. Gentle boiling was maintained for 30 minutes, the beaker being covered with a watch glass to reduce evaporation. The beaker of sirup was then removed from the burner and placed on the table top to allow it to cool to room temperature. Any measurable loss of water by evaporation was then restored. The water thus restored was mixed thoroughly into the sirup, which was then sampled for analysis.

Analyses conducted on each sample of sirup consisted of a pH determination and an assay of the dextrose and levulose content. The pH values were obtained with a glass electrode, employing a Leeds and Northrup Potentiometer-Electrometer for making the measurements. For ascertaining the amount of dextrose and levulose present in the sirup, the iodine oxidation method described by Lothrop and Holmes (4) was employed. In this method it is suggested that any satisfactory procedure for determining total reducing sugar may be used. The writer chose to use the Lane-Eynon volumetric procedure (1).

B. Effect of concentration of solution on sucrose inversion. Five batches of invert sirup were prepared by using 1 lb. sucrose and varying amounts of water. The amounts of water used were 175 ml., 200 ml., 225 ml., 250 ml., and 300 ml. Tartaric acid was used as the catalyst. The sirups were boiled for 30 minutes in 1-liter Erlenmeyer flasks. Each flask was provided with a thermometer and a glass tube 7 mm. × 30 inches, which served as an air-cooled reflux condenser to minimize loss of moisture. Analyses for pH, dextrose content, and levulose content were made according to methods described under A.

C. Effect of boiling time on suucrose inversion. Three series of sirup were prepared in this phase of the study. In each series the sirups were prepared by boiling together 1 lb. sucrose and 0.44 lb. of water, using tartaric acid as the catalyst.

In series I, one batch of sirup was boiled under a reflux condenser. Samples for analysis were withdrawn at the end of 20 minutes, 30 minutes, and 40 minutes.

In series II, three separate batches of sirup were prepared, using the same proportions of ingredients as in series I. The three batches were boiled under reflux condensers for 15 minutes, 30 minutes, and 45 minutes, respectively.

Series III was a repetition and extension of Series II. Four batches of sirup were prepared by boiling for 15 minutes, 30 minutes, 45 minutes, and 60 minutes, respectively.

Analyses of all sirups for pH, dextrose content, and levulose content were made according to methods described under A.

D. Time, temperature, and pH as factors affecting sucrose inversion. Temperatures used in this phase of the study were 190° F. and 175° F. At 190° F. the sirups were held for 30 minutes, 45 minutes, 60 minutes, and 90 minutes. At 175° F., heating periods of 45 minutes, 60 minutes, 90 minutes, and 120 minutes were employed. Five or more batches of sirup were prepared at each temperature and holding time, the variable factor being pH. Two parallel series of sirups representing the above combinations of conditions were made (82 batches of sirup), using tartaric acid in one series and phosphoric acid in the other.

Analyses of all sirups for pH, dextrose content, and levulose content were made according to methods described under A.

RESULTS

The relationship between the kind and amount of acid used and the results of the inversion process is indicated by the data shown in table 1. In connection with the values for the "total reducing sugar" it might be pointed out that, by way of comparison, the sirup should theoretically contain 73.07 per cent total reducing sugar if the sucrose were completely inverted, and the resulting invert sugar completely recovered.

TABLE 1

Effect of kind of acid and pH on sucrose inversion

Acid solution used	pH of sirup	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical sweetness index
		Tartaric acid			
ml.		*per cent*	*per cent*	*per cent*	
0.25	3.20	22.86	21.51	44.37	65.88
0.50	2.83	34.22	29.50	63.72	93.22
1.50	2.54	36.00	29.41	65.41	94.82
5.0	2.09	36.05	33.09	70.14	103.23
20.0	1.73	35.69	30.42	66.11	96.53
		Citric acid			
0.50	3.41	19.38	19.31	38.69	58.00
1.0	3.00	31.24	26.26	57.50	83.76
2.0	2.77	35.06	30.03	65.09	95.12
12.0	2.15	36.03	34.45	69.48	103.93
6 grams*	1.91	34.53	32.31	66.84	99.15
		Lactic acid			
0.20	3.16	23.86	22.04	45.90	67.94
0.75	2.68	34.47	32.24	66.71	98.95
1.50	2.45	35.85	30.64	66.49	97.13
3.0	2.23	36.61	32.83	69.44	102.27
6.0	2.00	35.86	33.41	69.27	102.68
14.0	1.79	35.41	31.51	66.92	98.43
		Acetic acid			
0.50	3.40	16.37	15.11	31.48	46.59
3.0	2.80	33.96	32.81	66.77	99.58
15.0	2.25	35.63	35.16	70.79	105.95
25.0	2.20	35.41	36.50	71.91	108.41
40.0	2.03	35.64	34.09	69.73	103.82
55.0	1.91	34.82	32.19	67.01	99.20
		Hydrochloric acid			
1.0	3.75	7.59	5.29	12.88	18.17
2.0	2.63	35.96	33.71	69.67	103.38
3.0	2.17	35.68	32.25	67.93	100.18
5.0	1.85	34.98	30.98	65.96	96.94
10.0	1.52	32.38	25.57	57.95	83.52
		Sulfuric acid			
0.75	3.20	16.84	17.57	34.41	51.98
1.00	2.71	32.12	32.91	65.03	97.94
1.50	2.43	35.66	33.97	69.63	103.60
2.50	2.03	35.35	33.36	68.71	102.07
5.0	1.65	34.34	30.54	64.88	95.42
		Phosphoric acid			
1.00	3.24	17.75	16.78	34.53	51.31
2.00	2.55	35.33	31.17	66.50	97.67
4.0	2.18	35.27	31.70	66.97	98.67
7.0	1.85	35.18	33.05	68.23	101.28
15.0	1.61	35.36	28.63	63.99	92.62

* Pure crystals.

TABLE 2

Effect of concentration of solution on sucrose inversion

Batch No.	Amount water per pound sucrose	pH		Dextrose in sirup			Levulose in sirup			Total reducing sugar in sirup			Theoretical Sweetness Index		
	ml.			*per cent*			*per cent*			*per cent*					
		A	B	A	B	Av.	A	B	Av.	A	B	Av.	A	B	Av.
1	175	2.10	2.05	35.27	35.66	35.47	28.91	30.87	29.89	64.18	66.53	65.36	93.09	97.40	95.25
2	200	2.11	2.07	35.23	35.96	35.60	32.59	30.20	31.40	67.82	66.16	66.99	100.41	96.36	98.39
3	225	2.13	2.09	35.28	35.96	35.62	32.21	32.20	32.21	67.49	68.16	67.83	99.70	100.36	100.04
4	250	2.18	2.13	35.60	36.09	35.85	33.21	33.96	33.59	68.81	70.05	69.43	102.02	104.01	103.02
5	300	2.22	2.18	35.32	36.22	35.77	34.55	31.84	33.20	69.87	68.06	68.97	104.42	99.90	102.17

A word of explanation is also necessary regarding the "Theoretical Sweetness Index" values tabulated in the last column of table 1. This is a purely arbitrary value which is the sum of the per cent total reducing sugar and per cent levulose in the invert sirup. It was believed that the total reducing sugar content alone, notwithstanding its importance, would not give a satisfactory sweetness value for comparing different methods of inverting sucrose. Theoretically at least, a high proportion of levulose in the invert sirup would be necessary to obtain maximum sweetness. For want of a better means, then, the "Theoretical Sweetness Index" was adopted for

TABLE 3

Effect of boiling time on sucrose inversion

Boiling time	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		(a)		
min.	%	%	%	
20	35.72	31.19	66.91	98.10
30	36.14	34.58	70.72	105.30
40	35.95	33.18	69.13	102.31
		(b)		
15	34.92	29.36	64.28	93.64
30	34.60	33.09	67.69	100.78
45	34.27	33.66	67.93	101.35
		(c)		
15	34.13	35.20	69.33	104.53
30	35.84	32.72	68.56	101.28
45	35.69	31.11	66.80	97.91
60	35.45	30.87	66.32	97.19

Note: The pH of the above sirups was approximately 2.1 to 2.2.

the purpose of permitting a quantitative comparison to be made of the theoretical sweetness of sirups produced by the various procedures considered in this paper.

In the commercial preparation of invert sirup 95 per cent inversion is considered satisfactory. If, then, 95 per cent of the sucrose is inverted and the resulting invert sugar consists of equal parts of dextrose and levulose, the Theoretical Sweetness Index (TSI) would be $(0.95 \times 73.07) + \frac{0.95 \times 73.07}{2}$, or 104.13. For purposes of comparison a TSI of 104 appears to be a reasonable standard value.

The effect of sugar concentration on the inversion process is indicated by the data given in table 2. Two series of sirups, A and B, were prepared

separately in the endeavor to obtain more representative results. In order to simplify a comparison of the five batches of sirup, all analytical values for dextrose, levulose, and total reducing sugar were adjusted to an equivalent water content. All data in table 2 were recalculated on the basis of 200 ml. water (Batch No. 2), so that the figures would be comparable to those given in table 1.

In table 3 are given the data showing the effect of the length of the boiling period on the inversion of sucrose. In this table parts (a), (b), and (c) correspond to Series I, II, and III, respectively, as outlined in *Experimental Procedure, C.*

Because of the large quantity of data secured in the investigation of the effect of temperature and time on sucrose inversion, these results are presented in four parts, namely, tables 4, 5, 6, and 7.

TABLE 4

Effect of pH and heating time on inversion of sucrose at 190° F. using tartaric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		30-minute heating		
	%	%	%	
3.37	3.16	2.94	6.10	9.04
3.03	5.58	3.86	9.44	13.30
2.58	18.15	14.07	32.22	46.29
2.11	30.35	28.53	58.88	87.41
1.67	32.88	37.12	70.00	107.12
		45-minute heating		
3.18	6.69	3.52	10.21	13.73
2.97	11.90	7.08	18.98	26.06
2.48	25.11	16.04	41.15	57.19
2.06	34.10	32.05	66.15	98.20
1.67	35.45	34.57	70.02	104.59
		60-minute heating		
3.21	9.44	3.88	13.32	17.20
2.92	15.62	7.92	23.54	31.46
2.46	28.60	20.98	49.58	70.56
2.07	34.65	29.29	63.94	93.23
1.67	35.55	33.71	69.26	102.97
		90-minute heating		
3.31	9.69	6.44	16.13	22.57
2.92	18.90	14.78	33.68	48.46
2.49	31.20	28.32	59.52	87.84
2.08	35.54	34.74	70.28	105.02
1.70	35.69	30.19	65.88	96.07

DISCUSSION

A. Effect of kind of acid and pH on sucrose inversion. It will be helpful, in studying the data presented in the foregoing tables, to keep in mind the

results which one is supposed to obtain by inverting sucrose. By a perfect inversion of sucrose solutions of the concentration used in this study, theoretically one should obtain a sirup containing 73.07 per cent total reducing sugar, half of which (36.54 per cent of the sirup) would be levulose and the other half dextrose. The Theoretical Sweetness Index would be 109.61.

Examination of table 1 reveals at once that this accomplishment was not realized in one of the 37 batches of sirup. The nearest approach (98.4 per

TABLE 5

Effect of pH and heating time on inversion of sucrose at 190° F. using phosphoric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		30-minute heating		
	%	%	%	
3.43	4.30	1.19	5.49	6.68
2.68	15.49	8.96	24.45	33.41
2.13	30.42	23.21	53.63	76.84
1.87	34.39	27.76	62.15	89.91
1.63	35.36	34.71	70.07	104.78
		45-minute heating		
3.53	4.06	2.39	6.45	8.84
2.72	18.17	12.93	31.10	44.03
2.13	32.37	24.94	57.31	82.25
1.88	34.88	34.03	68.91	102.94
1.63	36.43	33.67	70.10	103.77
		60-minute heating		
3.52	4.82	2.85	7.67	10.52
2.75	16.61	17.04	33.65	50.69
2.13	33.45	26.53	59.98	86.51
1.89	35.35	30.18	65.53	95.71
1.67	35.43	35.37	70.80	106.17
		90-minute heating		
3.53	6.26	7.43	13.69	21.12
2.72	22.59	23.60	46.19	69.79
2.17	34.13	35.47	69.70	105.17
1.92	35.56	35.34	70.90	106.24
1.67	35.46	35.73	71.19	106.92

cent) to "complete" inversion was in the batch of sirup prepared with acetic acid at a pH of 2.20. Likewise the highest TSI was obtained in this batch. Ironically enough, the acetic acid sirups were the only ones which were not usable in ice cream because of the undesirable penetrating aroma of the acid.

It will be noted that in ¾ of the 37 batches of sirup the quantity of dextrose found exceeded that of levulose—in many cases by an appreciable margin. The levulose content also appears to be somewhat more variable than the dextrose content. This relationship accounts for the fact that, in each series of sirups, the highest total reducing sugar content and the highest

TSI are usually found in that batch which contains the largest quantity of levulose.

Judging from the data presented in table 1 it appears that the optimum pH for inverting sucrose at the boiling temperature is within the range 2.0 to 2.6, and that the stronger the acid being used, the higher the pH should be. Careful study of the data in tables 1, 4, 5, 6, and 7 suggests that the pH

TABLE 6

Effect of pH and heating time on inversion of sucrose at 175° F. using tartaric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		45-minute heating		
	%	%	%	
3.16	4.85	8.00	12.85	20.85
2.86	8.52	15.36	23.88	39.24
2.43	18.03	30.35	48.38	78.73
2.13	25.87	34.12	59.99	94.11
1.68	35.20	36.10	71.30	107.40
		60-minute heating		
3.20	5.94	7.43	13.37	20.80
2.89	10.56	12.85	23.41	36.26
2.46	20.89	21.90	42.79	64.69
2.08	31.63	32.76	64.39	97.15
1.74	35.26	35.75	71.01	106.76
		90-minute heating		
3.53	3.01	4.17	7.18	11.35
3.16	6.72	10.42	17.14	27.56
2.60	17.06	26.78	43.84	70.62
2.16	30.46	35.46	65.92	101.38
1.73	35.43	36.03	71.46	107.49
		120-minute heating		
3.47	4.25	5.63	9.88	15.51
3.13	8.03	11.46	19.49	30.95
2.55	22.31	27.23	49.54	76.77
2.10	32.77	36.58	69.35	105.93
1.71	35.47	36.72	72.19	108.91

of the sirup is a very important factor in determining the consequences of the inversion process.

At this point it should be mentioned that in each acid series reported in table 1, what appears to be the optimum pH is merely the optimum of the pH values studied. Taking the citric acid series as an example, a pH of approximately 2.15 may or may not be optimum. All that is known from the data available is that somewhere within the pH range 2.77 to 1.91 one should obtain best results. Further investigation along this line would undoubtedly yield interesting information.

The practical results of this phase of the study would seem to be that a reasonably good invert sirup can be made by the boiling procedure, using any of the acids employed in these experiments (except acetic acid), assuming that proper control of pH in the sirup is exercised.

B. Effect of concentration of solution on sucrose inversion. It has been recommended in the past that invert sirup for ice cream manufacture be prepared from a sucrose solution consisting of 100 pounds of sucrose and

TABLE 7

Effect of pH and heating time on inversion of sucrose at 175° F. using phosphoric acid

pH	Dextrose in sirup	Levulose in sirup	Total reducing sugar in sirup	Theoretical Sweetness Index
		45-minute heating		
	%	%	%	
3.46	2.02	3.51	5.53	9.04
2.75	6.49	15.39	21.88	37.27
2.17	20.35	36.02	56.37	92.39
1.91	28.67	30.30	58.97	89.27
1.82	29.28	32.06	61.34	93.40
1.68	33.24	33.43	66.67	100.10
		60-minute heating		
3.57	2.32	3.79	6.11	9.90
2.77	9.69	18.89	28.58	47.47
2.18	21.21	22.69	43.90	66.59
1.89	30.36	30.21	60.57	90.78
1.65	34.26	34.05	68.31	102.36
		90-minute heating		
3.48	3.05	4.77	7.82	12.59
2.69	12.68	20.44	33.12	53.56
2.13	26.52	35.89	62.41	98.30
1.85	32.89	36.28	69.17	105.45
1.68	35.18	36.70	71.88	108.58
		120-minute heating		
3.74	2.51	3.26	5.77	9.03
2.85	12.18	19.04	31.22	50.26
2.17	29.00	35.63	64.63	100.26
1.90	33.97	33.21	67.18	100.39
1.69	35.15	34.94	70.09	105.03

44 pounds of water. This ratio of sugar to water was no doubt selected so that one pound of invert could be used to replace one pound of sucrose in the ice cream mix, thus simplifying mix calculations.

The TSI values in table 2 show that as the proportion of water was increased from 27.5 per cent (Batch 1) to 35.1 per cent (Batch 4) there was a gradual improvement in the inversion process. This was due primarily to an increase in the levulose content, for there was no significant difference among the several batches of sirup with respect to dextrose content. It is

questionable whether the better inversion in the less concentrated solution is of much practical importance, inasmuch as the disadvantage of having to use a more dilute sirup would at least partly offset the advantage of slightly better inversion.

C. Effect of boiling time on sucrose inversion. It is to be noted that there is some difference in the results obtained with the three series of samples reported in this phase of the study. The data in table 3a (Series I) indicate that 30 minutes boiling time is definitely superior to either 20 minutes or 40 minutes. Either 30 minutes or 45 minutes boiling was much superior to 15 minutes in the Series II sirups. The advantage of boiling 45 minutes as compared with 30 minutes is probably too small in this instance to be considered significant. In the Series III trials the 15-minute period seemed to give somewhat better results than the three longer boiling periods. It is difficult to account for what appears to be an anomalous behavior of the inversion process in this series.

The data of table 3 substantiate previous observations that the variations in the TSI are principally the result of variations in the levulose content of the sirups. The dextrose content of the sirups prepared by the different boiling periods exhibit a remarkable degree of uniformity.

D. Time, temperature, and pH as factors affecting sucrose inversion. A number of interesting observations may be gleaned from the data presented in tables 4–7, some of which may only be of theoretical importance.

Of considerable significance is the fact, disclosed by the data in these tables, that the proper pH is essential for bringing about suitable inversion at the lower temperatures used in this part of the investigation. For instance it is seen that even in those sirups which were heated for two hours at 175° F., there was little inversion except when the pH values were approximately 2.5 or below. Reasonably complete inversion was not obtained until the pH was reduced to about 2.1 or below. Whereas the optimum pH was about 2.0–2.6 for the boiled sirups, best results were obtained in the sirups inverted at 175° F. and 190° F. when the pH was adjusted to approximately 1.65–1.75.

It was suggested in the discussion of the boiled sirups that the apparent optimum hydrogen ion concentration in each series could only be considered as the optimum of the conditions represented. It is logical to believe that this is also true of the sirups prepared at the lower temperatures. In 15 of the 16 series of sirups inverted at 175° F. or 190° F., the highest TSI values were obtained in the batches of sirups having the lowest pH values. From this observation it seems that the present investigation could profitably be extended to determine more definitely the optimum hydrogen ion concentration for sirups to be inverted at temperatures below boiling.

On the basis of the results shown in tables 1, 4, 5, 6, and 7, there is a definite relationship among three factors, namely, temperature of heating,

time of heating, and hydrogen ion concentration. It appears that when any one of these is increased, either or both of the others may be decreased.

The levulose content of the sirups prepared at 190° F. is seen to be lower than the dextrose content. This is especially pronounced in the batches with high pH, where the total reducing sugar content is quite low. This dextrose-levulose relationship is reversed in the sirups heated at 175° F. (see tables 6 and 7). The true explanation of this is not known, although Jordan's (3) statement that levulose will begin to caramelize if the solution is not cooled "immediately after the reaction has finished" suggests a lower heat stability of levulose as compared with dextrose. This may appear to be a reasonable explanation for the composition of the sirups inverted at 190° F., but it does not adequately account for the composition of sirups containing more levulose than dextrose, a condition which prevailed in nearly all batches heated only to 175° F.

By way of summarizing the practical results of the experiments reported in Tables 4 to 7 it may be said that either 190° F. or 175° F. produced satisfactory invert sirups, provided the length of the heating period and the pH were controlled properly. It has also been demonstrated that phosphoric acid was as good a catalyst as the more expensive tartaric acid.

SUMMARY AND CONCLUSIONS

The commercial "inversion" of sucrose by heating its water solution in the presence of acid seldom produces a sirup identical in composition with that which theoretically should be obtained.

Invert sirups prepared by boiling and by heating at 190° F. contained more dextrose than levulose. Sirups prepared by heating at 175° F. contained more levulose than dextrose.

On the basis of data obtained in the present investigation, it was found that the optimum acidity for inverting sucrose at boiling temperature was represented by a pH range of 2.0 to 2.6. With the lower heating temperatures (175° F. and 190° F.), pH values of approximately 1.65 to 1.75 were necessary to obtain satisfactory inversion.

Preliminary studies indicate that any of the following acids could be used for making invert sirup: tartaric, citric, lactic, hydrochloric, sulfuric, phosphoric.

There was a slight improvement in the inversion process as the water content of the uninverted sirup was increased from 27.5 per cent to 35.1 per cent.

Although some discrepancy was noted in the results secured with different boiling periods, the 30-minute period seemed to represent the best average.

Three factors influencing the inversion of sucrose appear to be positively interrelated. These are temperature of heating, time of heating, and hy-

drogen ion concentration. When any one or two of these is increased the other one or two may be decreased.

Invert sirup of good quality was prepared at 175° F. and 190° F. by holding the sirup at these temperatures for 45 to 90 minutes. The exact temperature and time were found to be less critical factors than pH.

Sirup prepared with phosphoric acid was found to be as good, on a TSI basis, as sirup prepared with tartaric acid.

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STUDIES OF THE CARBOHYDRATE METABOLISM OF MAMMARY GLAND TISSUE IN VITRO. II. THE METABOLISM OF CITRIC ACID AND B–HYDROXYBUTYRIC ACID IN TISSUE SLICES[1]

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While it has long been known that normal milk contains relatively large amounts of citric acid, no reference has been found as to its source. Analysis of simultaneously drawn arterial and mammary vein bloods failed to reveal any significant difference in the values for this substance indicating very little or no uptake of citric acid by the mammary gland from the blood.

To account for all of the citric acid found in milk by uptake from the blood would practically exhaust the latter of this substance in its course through the gland since it is several hundred times as concentrated in the milk as in blood. This fact suggests that citric acid is synthesized in the mammary gland and gives rise to speculation as to its blood precursors and the mode of synthesis.

Shaw and Knodt (4) working with intact cows and Shaw and Petersen (5) using the perfusion technique have demonstrated that the active mammary gland uses large quantities of β-hydroxybutyric acid. The fate of this substance in the gland, however, is only speculative. The possibility of its serving as a precursor of citric acid is suggested.

The investigation was undertaken in an attempt to ascertain whether or not citric acid is synthesized by the mammary gland and if so to determine the probable blood precursors as well as to endeavor to ascertain the probable fate of β-hydroxybutyric acid in milk formation.

EXPERIMENTAL

Because of the success experienced in a study of lactose formation by the use of tissue slices (2), this method of attack was suggested. The slices were prepared and incubated as described by Knodt and Petersen (2). In the study of citric acid formation a series of six experiments were run with each of the following additions: 1 per cent glucose, 0.5 per cent glucose and 0.5 per cent lactic acid, 1 per cent lactic acid, 1 per cent citric acid, 1 per cent pyruvic acid, 1 per cent maltose and 1 per cent glycogen. All experiments were run in duplicate and controls without any additions were also run with

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each series. The percentage of citric acid is expressed on the basis of tissue weight. Citric acid was determined by the methed of Pucher *et al.* (3).

The study of β-hydroxybutyric acid metabolism was made in another series of ten experiments in each of which in addition to the controls duplicates with additions of 50, 100, and 200 mg. β-hydroxybutyric to 25 gm. tissue were incubated for 6 hours at 37.5° C. with oxygen aeration every 2 hours. In addition to citric acid determinations, analysis for β-hydroxybutyric acid, acetoacetic acid and acetone were made according to the method of Barnes and Wick (1). The acetoacetic acid and acetone were determined as acetone.

RESULTS

The results for incubation of 6 hours duration of controls and samples to which were added 1 per cent glucose, 0.5 per cent glucose and 0.5 per cent lactic acid, 1 per cent lactic acid, 1 per cent citric acid, 1 per cent pyruvic acid, 1 per cent maltose and 1 per cent glycogen are presented in table 1. It will be noted that in each experiment there is a significant increase in citric acid during incubation in the controls to which no additions were made. On the average, this increase was 9.19 mg. per cent from 17.53 to 26.72 mg. per cent.

Greater increases in citric acid were observed in every experiment where additions were made with the exception of those in which large amounts of citric acid were added. In the latter incubation there was a significant decrease in citric acid.

Results of the 10 experiments in which 50, 100, and 200 mg. β-hydroxybutyric acid were added are presented in the tables 2 and 3. In table 1 it will be seen that in all experiments a significant decrease in β-hydroxybutyric acid resulted and significant amounts of acetone and acetoacetic acid developed. No acetone bodies could be detected in the tissue without the addition of the β-hydroxybutyric acid either before or after incubation. It will also be noted that the use of β-hydroxybutyric acid increased with increments in the amounts added. The losses were 17.86, 33.09 and 92.4 mg., respectively for 50, 100, and 200 mg. additions of β-hydroxybutyric acid.

The amounts of acetone and acetoacetic acid formed during the incubation increased with increments in added β-hydroxybutyric acid. The average values being 5.29, 6.82 and 9.50, respectively, for 50, 100, and 200 mg. additions.

The effect of β-hydroxybutyric acid additions to incubated tissue slices upon citric acid formation is presented in table 3. Only slight increases in citric acid above those for the incubated controls are noted. The average values are 9.56, 10.57, 10.78 and 11.08 mg. respectively for the control and those to which, 50, 100 and 200 mg. additions of β-hydroxybutyric acid were made.

TABLE 1

The metabolism of citric acid during incubation of mammary gland tissue slices*

Experiment number	Before incubation		After incubation							
	Control	Control & 1% citric acid	Control	Glucose	0.5% glucose & 0.5% lactic acid	1% lactic acid	1% citric acid	1% pyruvic acid	1% maltose	1% glycogen
1	16.98	1011.8	25.95	47.80	35.46	34.57	798.0	40.93	31.41	29.42
2	17.28	1020.8	24.85	37.88	32.15	28.62	834.2	30.76	27.52	29.73
3	14.27	1021.8	27.92	48.05	51.26	30.20	885.5	34.47	35.21	32.17
4	23.16	1038.0	27.51	45.33	37.25	32.27	882.4	33.29	29.30	30.54
5	15.29	1016.0	32.49	50.21	38.94	34.76	860.7	41.62	37.21	43.85
6	18.20	1037.1	21.60	34.84	32.47	26.98	829.1	31.94	26.14	30.30
Average	17.53	1024.2	26.72	44.02	37.92	31.23	848.3	35.50	31.13	32.67

* Expressed as mg.% present in tissue.

TABLE 2

Utilization of β-hydroxybutyric acid by mammary gland tissue slices

Mg. β-hydroxybutyric acid added	1	2	3	4	5	6	7	8	9	10	Avg.
Mg. β-hydroxybutyric acid used by 25 gm. of tissue slices in 6 hrs.											
50	31.80	42.25	27.41	27.85	38.06	36.53	33.80	30.60	24.68	28.84	32.14
100	64.26	69.95	61.97	63.80	73.42	69.73	70.15	65.45	62.65	67.73	66.91
200	107.8	139.6	98.90	94.40	116.7	107.5	105.0	112.0	96.20	108.2	108.6
Mg. acetone and acetoacetic acid formed (as acetone)											
50	6.10	8.25	5.41	2.01	4.55	4.29	5.20	6.27	4.75	6.05	5.29
100	7.00	9.15	6.77	5.27	7.46	6.28	6.75	6.80	5.80	6.94	6.82
200	8.80	10.20	8.35	8.33	10.08	8.95	8.21	8.54	7.23	7.70	9.50

DISCUSSION OF RESULTS

It is significant that active mammary tissue slices incubated without any additions form citric acid. In all experiments, this increase was observed which amounted to 52.4 per cent. Since some time elapsed between slaughter and determination of citric acid in the tissue, it is reasonable to assume some formation had taken place in the interim. The citric acid formation under these conditions must be from materials present in the tissue presumably from the glycogen and other carbohydrates. This observation establishes the fact that the mammary gland can synthesize citric acid.

The increments of citric acid after incubation, over the controls following the addition of glucose, lactic acid, pyruvic acid, maltose and glycogen indicate that these substances can serve as precursors for citric acid forma-

TABLE 3

The effect of added β-hydroxybutyric acid upon citric acid metabolism

Experiments	Citric acid present (expressed as mg. present)				
	Control before incubation	After incubation β-hydroxybutyric acid added			
		Control	50 mg.	100 mg.	200 mg.
1	4.61	7.71	9.45	9.59	9.85
2	4.41	7.40	9.54	9.51	9.92
3	5.54	8.81	10.26	10.48	10.88
4	7.97	10.79	11.26	11.63	12.35
5	7.23	10.03	12.12	12.32	12.54
6	10.12	12.84	13.54	13.98	13.98
7	6.53	8.91	9.48	9.61	9.97
8	6.24	8.68	8.82	8.98	9.32
9	7.79	9.71	10.01	10.40	10.85
10	8.57	10.75	11.19	11.33	11.30
Average	6.90	9.56	10.57	10.78	11.08

tion in the mammary gland. The possibility, however, remains that these substances acted indirectly to increase the citric acid by stimulating other metabolic processes which in turn increase citric acid formation. It may be significant that the largest increases in citric acid are observed where glucose was added either alone or in combination with lactic acid. The significant average decrease of 175.9 mg. on incubation of tissue slices containing an average of 1024.2 mg. per cent citric acid indicates that the reaction can become reversed when the concentration becomes too great.

The addition of β-hydroxybutyric acid to incubated tissue slices confirms the observations of Shaw and Knodt (4), and Shaw and Petersen (5) in that the use of this substance by the mammary gland increases with its concentration. The observed increments in acetone and acetoacetic acid formation with increases in β-hydroxybutyric acid additions indicates that part of the latter is converted into these substances. Assuming a given amount

of β-hydroxybutyric acid is converted into an equal amount of acetone and acetoacetic acid approximately 1/3, 1/5, and 1/10 of the β-hydroxybutyric acid used during incubation can be accounted for by this conversion respectively when 50, 100 and 200 mg. were added.

While increases in citric acid formation was observed with each increment of β-hydroxybutyric acid except in two instances out of the 30 observations the differences are so small as to be of questionable significance. At least in no instance was the citric acid formation comparable to that observed when glucose, lactic acid, pyruvic acid, maltose or glycogen were added.

SUMMARY AND CONCLUSIONS

A series of six experiments is reported on the formation of citric acid in incubated mammary tissue slices with and without the addition of glucose, glucose plus lactic acid, lactic acid, pyruvic acid, citric acid, maltose and glycogen. Ten other experiments on the fate of β-hydroxybutyric acid in mammary tissue slices is also reported.

It is shown that the mammary gland can synthesize citric acid from substances in the tissue.

Addition to the slices of glucose, lactic acid, pyruvic acid, maltose or glycogen increased citric acid formation indicating that these substances may serve as precursors.

When large amounts of citric acid were added its degradation was observed upon incubation.

Increments in added β-hydroxybutyric acid is used in increasing amounts by the incubated tissue slices.

Part of the added β-hydroxybutyric acid is converted into acetone and acetoacetic acid. The amount converted decreases with increased concentration and use.

Conversion of β-hydroxybutyric acid into citric acid by the mammary gland is questionable.

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STUDIES OF THE CARBOHYDRATE METABOLISM OF MAMMARY GLAND TISSUE IN VITRO. III. GLYCOGEN AS AN INTERMEDIARY IN THE FORMATION OF LACTOSE[1]

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Although the mammary gland has been shown to take up the carbohydrate portion of glycoproteins (17), to use lactic acid under certain conditions (6, 15) and to form lactose from glucose, maltose and glycogen in *in vitro* experiments (10, 11), blood glucose is considered to be the chief precursor of milk sugar (2, 7, 8, 9, 13, 19). The reduced milk secretion and lactose concentration following insulin administration (3, 5, 14) has been attributed to the resulting hypoglycemia suggesting that glucose is directly converted into the milk sugar in the mammary gland.

Reports by Petersen *et al.* (15) that lactating glands contain relatively large amounts, and non-active glands small amounts of glycogen raises the question of the rôle played by this substance in lactose synthesis. Demonstrations by Knodt and Petersen (12) that glycogen can be converted into lactose, suggest the possibility of the various blood precursors being first converted into glycogen but does not prove the point as the glycogen might serve merely as a carbohydrate reservoir to be drawn upon in case of inadequate blood precursors.

With this postulation the lowered lactose concentration following insulin administration would be accounted for by the blocking of glycogen degradation rather than by the resulting hypoglycemia.

An obvious test of the validity of this hypothesis would be maintaining the normal or increasing the blood glucose level with the administration of insulin, and noting the effect upon the lactose concentration in the milk and glycogen concentration in the tissue. If the lactose concentration should become less and the glycogen stores increase with insulin administration in the presence of adequate amounts of glucose such would support the hypothesis.

Since it would be difficult to maintain the above stipulated conditions in the intact animal and since normal milk secretion can be obtained in the perfused isolated bovine gland, where these conditions can easily be met, perfusion became the method of choice in this study.

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EXPERIMENTAL

A total of 17 perfusions were performed. In a series of 10 perfusions the additions of glucose were of the magnitude calculated to maintain normal blood levels. One half of each gland served as control while to the other half 160 units of insulin were added. In a series of 7 perfusions, large excesses of glucose were added, to produce a state of hyperglycemia, and from 120 to 160 units of insulin.

The perfusions were performed with a modified apparatus similar to that described by Petersen *et al.* (16) on one half of apparently normal lactating udders obtained from local abattoirs. Immediately after the perfusion was started, the glands were emptied of milk by injection of 10 units of oxytocin. Insulin was then added to the blood. In all experiments the glands were emptied by the use of oxytocin after 1 hour of perfusion and then hourly or every 2 hours up to 4 or 5 hours. The amounts of milk obtained at each milking was ascertained and analyzed for lactose.

Lactose was determined on each sample of milk according to the method of Bierman and Doan (1). Blood glucose was determined according to the methods of Schaeffer and Somogyi (18) and tissue glycogen according to Good, Kramer and Somogyi (4). Chlorazol fast pink was used in the blood as an anticoagulant. The insulin used was "Regular Insulin" produced by Eli Lilly and Company.

RESULTS

In table 1 are presented the results of perfusing with approximately normal amounts of blood glucose. In the first 5 experiments (Group I) insulin was not administered. To each of the second group of five experiments (Group II) 160 units of insulin was added. While there is a small progressive drop in lactose content with the successive milkings in the experiments where insulin was not administered in the experiments where insulin was added the drop is precipitous. In the former the original milk contained 3.77 per cent and ended after 5 hours of perfusion with 2.84 per cent of lactose while in the insulinized glands the respective values were 3.62 and 0.65 per cent.

The glycogen contents of the glands of the two groups were essentially the same at the beginning of the experiment, averaging 22.52 and 23.32 mg. per cent, respectively, for Group I and Group II. Although perfusion for 5 hours increased the glycogen content of the glands in both groups the average final values were approximately 2.5 times as high in the glands that were insulinized as in the controls. The blood glucose levels at the end of five hours of perfusion varied in both groups to such an extent as to preclude conclusions as to trends.

There is great variability within each group as to the quantities of milk obtained at the different milkings. No significant difference between the two

TABLE 1

The effect of insulin upon lactose and glycogen formation in the perfused mammary gland with normal blood glucose

Perfusion number	Milks										Final blood glucose	Glucose added	Insulin added
	Original		0–1 hour		1–3 hours		3–5 hours		Tissue				
	Amount	Lactose	Amount	Lactose	Amount	Lactose	Amount	Lactose	Glycogen original	Final			
Group I. Non-insulinized glands													
	ml.	%	*ml.*	%	*ml.*	%	*ml.*	%	*mg.%*	*mg.%*	*mg.%*	*gm.*	*units*
197	1400	3.70	500	3.38	330	3.09	290	2.99	26.05	62.50	22.50	5	0
198	1400	4.02	760	4.11	370	3.85	280	3.60	19.75	42.09	68.55	10	0
201	4460	3.58	460	3.67	270	3.08	220	2.90	23.17	65.43	61.60	10	0
202	1560	3.82	530	3.43	310	3.27	265	2.77	22.50	69.20	47.33	10	0
204	880	3.72	980	3.20	340	2.85	290	1.94	21.13	28.05	41.30	10	0
Ave.	1940	3.77	646	3.56	324	3.23	269	2.84	22.52	53.45	48.26	9	.....
Group II. Insulinized glands													
196	220	3.82	820	3.19	350	1.35	210	0.46	20.35	116.2	55.00	5	160
199	1540	3.70	850	3.74	195	1.86	45	0.42	21.44	145.7	48.35	10	160
200	1400	3.54	240	3.44	360	1.69	120	0.90	18.58	187.8	43.07	10	160
203	1090	3.47	240	2.65	380	1.58	200	0.67	25.00	92.2	57.30	10	160
205	760	3.58	1340	2.04	650	1.46	120	0.79	31.25	127.0	106.8	10	160
Ave.	1002	3.62	698	3.01	387	1.59	139	0.65	23.32	133.8	62.1	9	160

TABLE 2

The effect of insulin upon lactose and glycogen formation in perfused bovine mammary glands with large amounts of added glucose

Perfusion number	Milks										Final tissue glycogen	Glucose added	Insulin added
	Original		1 hour		2 hours		4 hours		5 hours				
	Amount	Lactose	Amount	Lactose	Amount	Lactose	Amount	Lactose	Amount	Lactose			
	ml.	%	*ml.*	%	*ml.*	%	*ml.*	%	*ml.*	%	*mg.%*	*gm.*	*units*
189	520	4.32	300	3.16	260	2.77	125	0.75	80	0.56	372.0	37.8	130
190	760	4.06	295	2.18	740	2.18	560	0.92	....	.....	158.6	37.8	120
191	1090	4.51	510	3.08	290	1.53	170	0.85	.....	.....	259.7	100.0	160
192	1260	3.94	320	3.07	250	1.95	210	1.04	.....	.....	288.0	50.0	160
193	1050	3.96	560	3.36	320	2.07	300	0.73	.....	.....	214.2	50.0	160
194	850	3.77	*	.....	620	2.38	230	1.31	60	0.49	.....	60.0	160
195	1040	4.12	*	.....	640	1.75	280	0.63	.....	.....	.....	60.0	160
Ave.	939	4.10	397	2.97	446	2.09	268	0.89	70	0.53	258.5	56.5	.....

* Not milked until 2 hours after beginning of perfusion. Final blood glucose determined on perfusions 194 and 195 were 272.4 and 341.0 mg. per cent respectively.

groups is observed until the last milking—after five hours of perfusion. Here the average quantity of 139 ml. of the insulinized group as compared to 269 ml. for the controls is significantly lower.

The results of seven experiments, all insulinized, to which large quantities of glucose were added, are presented in table 2. In two cases only was the glucose level in the blood determined revealing 272.4 and 341.0 mg. concentration for perfusions 194 and 195, respectively. From the observations made when 5 and 10 gm. of glucose were added it is certain that in all seven experiments a marked hyperglycemia was maintained throughout the experiments.

Five of the experiments were discontinued at the end of four hours while others were conducted for a longer period of time. At that time the lactose concentration in the milk had dropped from an average of 4.1 per cent at the beginning to 0.89 per cent. For the two experiments continued for five hours the lactose dropped to 0.56 and 0.49 per cent.

The glycogen concentration in the gland tissue was determined only in the first five experiments. In these the average concentration was 258.5 mg. per cent as contrasted to an average of 113.8 mg. per cent in the five previously cited experiments in which insulin was administered and the blood glucose was maintained at normal levels. The difference is of greater significance than the data indicates because of the shorter duration of the perfusions with high blood glucose. Four of the five in the latter were perfused only four hours, while in the low blood glucose group all were perfused five hours. Significantly the gland perfused five hours had by far the greatest glycogen concentration—372.0 mg. per cent.

DISCUSSION OF RESULTS

It is clear from the data presented that under the conditions of these experiments insulin decreased lactose formation, which was observable in the milking one hour after its administration. Just when and to what extent lactose formation was inhibited following the administration of insulin cannot be ascertained from these data because of the indeterminable amounts of residual milk remaining in the ducts and alveoli following each attempted evacuation by the use of oxytocin. It is reasonable to suppose, however, that the residual milk was not quantitatively sufficient to account for a major portion of the lactose found in the milks following insulin administration and therefore the inhibitory action of the insulin developed more or less gradually.

It is also clear that glycogen contents of the insulinized glands is significantly higher than the controls when perfused with bloods with normal glucose levels. This increase in glycogen can be accounted for by either or both of two postulates—that insulin facilitated the conversion of glucose into glycogen or it merely blocks glycogen breakdown without affecting the

rate of its formation. Unfortunately the data taken are not adequate to conclusively settle the question, but there is some indication that the insulin action is mainly that of blocking the breakdown of glycogen rather than accelerating its formation in the mammary gland.

Although weights were not taken and there was considerable variation in the sizes of glands used in the experiments, but since efforts were made to pair the glands used in Groups I and II it is fair to assume that the average weights were approximately the same for the two groups. From the data in table 1 it is calculated that 12.75 gm. more lactose was secreted in the milks obtained during the perfusion from the controls than from the insulinized glands. The insulinized glands contained 70.4 mg. per cent more tissue glycogen than the controls. To account for the equivalent of 12.74 mg. lactose in additional glycogen stores of 70.4 mg. per cent would require 18.2 kilos of gland tissue which exceeds by far the average weight of the glands used.

It is conservatively estimated that no gland used exceeded 10 kilos in weight and the average would be considerably less.

A second support for this postulate is found in the glucose concentration in the bloods at the termination of the experiments which shows no significant difference between the controls and the insulin-treated gland. If insulin should accelerate glycogen formation from glucose then the blood glucose levels should be lower in the experiments where insulin was used.

In table 2 it is seen that when the blood glucose is unusually high with insulin the glycogen deposition in the tissue becomes proportionately higher, an average of 258.5 mg. per cent for 5 experiments with a high of 372.0 mg. per cent for 1 experiment. However, Knodt and Petersen (12) have reported an average glycogen content of 231.7 mg. per cent with a high of 514 mg. per cent in a series of 20 similar experiments except that insulin was not administered.

With these facts as a basis it is postulated that lactose may be formed in the mammary gland from the degradation of glycogen. In all of the experiments reported herein there has been sufficient or an excess of glucose in the blood to produce milk with normal lactose content if that substance is converted directly into the milk sugar.

It is to be recognized that argument may be advanced that insulin interferes with other metabolic processes in the cell which prevents lactose formation. If such is the case it must be pointed out that such a metabolic disturbance must act preferentially on lactose formation, because, while there is evidence of decreased milk secretion from insulin administration, the depression of lactose synthesis is much greater.

The fact that the increased glycogen storage in the insulin experiments cannot account for the glucose spared by decreased lactose formation indicates a greater utilization of glucose for other purposes. While no attempt

is made here to review the extensive literature on the action of insulin it may be stated that the indicated greater use of glucose is controversial as is the theory of insulin blocking the degradation of glycogen and the stimulating effect on glycogen formation.

SUMMARY AND CONCLUSIONS

The results of a series of 17 perfusion experiments are reported in which the effects of insulin upon lactose formation and tissue glycogen storage were studied. The effects of insulin administered in a series of 5 experiments were compared with the results in 5 experiments where insulin was not added but glucose added to maintain normal or subnormal blood levels. In 7 experiments the effect of large additions of glucose together with insulin was studied.

Administration of 120 to 160 units of insulin markedly reduced the percentage of lactose in the milks even though the bloods contained adequate amounts of glucose for normal lactose production.

The accumulation of tissue glycogen was greater in the insulinized glands than in the controls.

Evidence is presented to indicate that the increase in tissue glycogen was not due to glycogenic properties of insulin but more likely to its blocking of glycogenolysis.

It is postulated that lactose is formed from degradation of glycogen and the lowered lactose production in experiments where insulin was administered is accounted for by a blockage of glycogen breakdown.

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To the Officers and Members of the American Dairy Science Association:

The Iowa State College joins with the Dairy organizations of Iowa in expressing great satisfaction that the American Dairy Science Association has decided to hold its 1946 annual meeting on our campus the eighteenth, nineteenth and twentieth of June.

As one of the great dairy states of America, we welcome you. We recall with pleasure your meeting at the College in 1930; and we are looking forward with even greater anticipation to your visit next June. We hope that the 1946 meeting of the Association will contribute materially to the success of the dairy industry of the nation.

Sincerely yours,
CHARLES E. FRILEY
President

FIRST CALL FOR PAPERS

Members who wish to give original papers at the annual meeting should send the titles to the Program Committee as promptly as convenient. All titles must reach the Program Committee before March 25, but early receipt of the titles is of real value in arranging the best program. All communications regarding general plans should be addressed to the chairman of the General Program Committee, but all titles of papers should be sent directly to the chairman of the Program Committee for the section before which the paper should be presented.

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ABSTRACTS OF LITERATURE

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The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

52. Control of Dust Contamination in Agar Pour Plate Analysis of Milk. G. C. Gambrell, Health Officer, Lexington, N. C., and Morris Ostrolenk, State Board of Health, Raleigh, N. C. Food Res. *10*. No. 4: 303. July–August, 1942.

A comparison of the degree of contamination of agar plates poured under unfavorable laboratory conditions with those poured under favorable conditions revealed a 17.2 per cent contamination in the first instance and a 3 per cent contamination in the second. F.J.D.

BUTTER

53 Vitamin A in Butter. Bureau of Dairy Indus., U.S.D.A. Mis. Pub. 571, July, 1945.

Twenty State Experiment Stations and the U. S. Bureau of Dairy Industry cooperated in this study to determine the vitamin A potency of butter produced and marketed in different seasons and different regions. The need for the study was indicated by the Food and Nutrition Board of the National Research Council. Some 3,500 samples of butter were analyzed from 14 states that produced 64 per cent of the butter in the United States.

"In all but one state there was a distinct difference between the vitamin A potency of butter produced under summer-feeding conditions. About 36 per cent of all the creamery butter is 'winter' butter, and it has an average vitamin A potency of approximately 11,200 International Units per pound. About 64 per cent of all creamery butter is 'summer' butter, and it has an average vitamin A potency of approximately 18,000 International Units per pound." The average for all creamery butter is about 15,000 I.U. per pound. When people eat the prewar per capita consumption of 18 pounds per year, butter furnishes 740 I.U. per capita per day or 14.8 per cent of daily requirements.

The effect of storage and handling on the vitamin A potency of butter was negligible, as reported by seven states that studied this point. In four states that studied butter on the retail markets, it was found that the vitamin A potency compared favorably with the vitamin A potency of the butter produced.

"Milk produced under winter-feed conditions has an average vitamin A potency of 1,140 International Units per quart (4 per cent fat basis) and that produced under average summer-feeding conditions has a potency of 1,800. The average potency of the total supply of milk which disappears annually for domestic consumption is 1,530 International Units per quart.

When the disappearance of milk for domestic consumption in all forms averages 1.052 quarts per capita per day, as it did in 1941, milk and milk products furnish the total population of this country about 1,600 International Units of vitamin A per capita per day, or nearly one-third of the recommended allowance."

The vitamin A potency of milk and butter is dependent upon the carotene content of the diet of the cows. A.C.D.

CHEESE

54 A Grating Type Cheese. C. D. DAHLE AND G. H. WATROUS, JR. Pa. State Col., Nat. Butter & Cheese Jour., *36,* No. 11: 44. Nov., 1945.

A new method of making a hard cheese for grating is described. The cheese develops a high, piquant flavor in approximately 10 months of curing. The characteristic flavor is caused by free fatty acids produced by the lipase of raw skim milk acting on the fat of homogenized, pasteurized, 20% cream. The cheese is made from a skim milk and cream mixture with 2% fat. The curd is made like stirred-curd cheddar but is cut finer, cooked to 115° F. and is only partially salted in the vat. It is pressed in Longhorn hoops and then is dry salted, oiled and rubbed with black pepper while curing at 60° F. and not over 80% relative humidity. The cheese contains about 27% fat, 27% moisture, 5 to 6% salt and has a pH of 5.2–5.7. W.V.P.

55 The Trend Toward Bigger Plants. ANONYMOUS. Natl. Butter & Cheese Jour., *36,* No. 9: 36. September, 1945.

Mr. Donald E. Hirsch in the article entitled "Farmers' Co-operatives and the Trend Toward Large Scale Dairy Plants," Misc. Rep. #80, Coop. Res. & Service Div., Farm Credit Administration, March, 1945, points out that large plants in general can utilize milk solids more efficiently than small plants. They achieve greater flexibility and have definite advantages in manufacturing, marketing and research not enjoyed by smaller organizations. Graphs are presented to illustrate the trends of increasing production per plant in the butter and chesse industries. W.V.P.

CHEMISTRY

56 A Simple Fluorometric Method for Thiamin in Milk. A. Z. HODSON, Res. Lab., Pet Milk Co., Greenville, Ill. Food Res. *10,* No. 4: 351. July-August, 1945.

A simplified fluorometric method for thiamin in milk based on the procedure of Hennessey and Cerecedo, but with the omission of the base-exchange step is presented. The method appears to be quick and reasonably accurate for milk and some other dairy products as judged by comparisons

with two biological methods employing *Phycomyces blakesleeanus* and *Streptococcus salivarius* S20B.

Results with the *Phycomyces* method were high for evaporated milk which was interpreted as indicating that the heat destruction of thiamin in this product is a simple cleavage into the pyrimidine and thiazole components which are biologically active for the mold. F.J.D.

ICE CREAM

57. What the Ice Cream Industry is Doing About Frozen Foods. ANONYMOUS. Ice Cream Field, *46,* No. 4: 16. 1945.

This is a report of a recent survey, attempting to ascertain the place of frozen foods in the ice cream industry. Various views are expressed in answer to questions regarding (1) the use of dealer ice cream cabinets, (2) installing display cabinets to merchandise frozen foods and package ice cream, (3) using the same delivery trucks and storage rooms for ice cream and frozen foods, (4) maintaining separate sales personnel for ice cream and frozen foods, and (5) restricting the number of frozen foods items distributed. W.C.C.

58. Merchandising Frozen Cooked Foods. DONALD K. TRESSLER, Technical Consultant, Ice Cream Field, *46,* No. 4: 68. 1945.

Some frozen cooked food specialty items appeared on the market prior to the war. During the war, several others were added to this list. Difficulty in distributing such products has been due to limited frozen food outlets, but chain grocery stores, super-markets, and department stores, it is stated, are now looking for other frozen cooked foods. Over forty frozen cooked foods are listed as now on the market.

The author cautions against freezing any cooked food, and recommends preliminary freezing and storage tests at various temperatures before attempting to produce such items on a commercial scale. Once a satisfactory recipe has been found, it should not be changed, unless the new recipe has proved satisfactory under thorough testing. The importance of chemical, as well as bacteriological controls, is stressed. W.C.C.

MILK

59. Use of Plate Type Heat Exchanges in High-Temperature, Short-Time Pasteurization. W. W. CAVANAUGH, York Corp., York, Pa. Natl. Butter & Cheese Jour., *36,* No. 9: 38. September, 1945.

This is a detailed description of the design, operation, control and care of this type of pasteurizer and such auxiliaries as storage tanks, filters, clarifiers, homogenizers and separators. W.V.P.

60. Wartime Changes in Milk Distribution and in the Consumption of Milk, Cream, Butter and Oleomargarine in Vermont. THURSTON M. ADAMS. Vt. Agr. Expt. Sta. Bul. 527. 30 pages. Oct., 1945.

The bulletin is based on information obtained for the most part from personal interviews in June, 1944, with 1,150 housewives in Burlington, Vermont, and in two nearby villages. Information contained in 112 usable replies to questionnaires sent to all milk dealers in Vermont is also included.

If a cent a quart could be saved over three-fourths of the housewives having doorstep delivery of milk would be willing to have every-other-day retail milk delivery continued after the war. In June, 1944, store purchases of milk in Burlington amounted to 42 per cent. Low rental areas purchased more milk at stores than those living in high rental areas. Family milk supply purchased at stores tended to increase with alternate-day delivery. Nearly all the milk dealers in Burlington and Rutland (Vermont's two largest cities) favored every-other-day delivery. In the smaller communities, many were opposed. Of the 112 dealers reporting, 53 per cent favored continuation, 38 per cent were opposed and 9 per cent were undecided at the time they replied. A savings resulted from a reduced number of distributors in the market aside from every-other-day delivery. The number of dealers serving the Burlington area declined about one-half between 1942 and 1944. This decline in the number of milk distributors is but an acceleration of a long-time trend in Vermont and in many other markets. Mileage savings result when fewer delivery trucks serve an area.

While milk consumption in the homes of the areas studied was high and had experienced a war-time increase, it was below the level recommended for adequate nutrition. While nearly all the families studied had fresh milk, only about one out of every five families purchased cream. About one half pound per person of butter and margarine were used weekly in the area studied. About two-thirds as much oleomargarine as butter were purchased by consumers in Burlington and Essex Junction, while in the small rural community of Shelburne, they were buying nearly twice as much oleo as butter. Butter consumption declined and oleomargarine consumption increased more under war-time conditions, in the areas studied than in the country as a whole. In a large measure those families who had shifted to the use of margarine when butter was in short supply had returned to the use of butter when it again became available.

Only a small portion of the new war-time users of margaine expressed a conviction that they would use the product in the post-war period. Nearly one-third of the new war-time users of margarine not planning to use it after the war indicated that while they preferred butter, they had no particular dislike for margarine. H.P.

61. Certain Defects in Market Milk By-products. R. J. RAMSEY, Ramsey Laboratories, Cleveland, Ohio. The Dairy World, *24*, No. 2: 21. July, 1945.

The author discusses, in some detail and from the viewpoint of the plant operator, many troublesome problems such as: poor keeping quality, lipase action, cream plug, cream line and air incorporation in the production of bottled cream; factors influencing deterioration of frozen cream; sediment, flavor, soapiness and curd tension of homogenized milk; production of superior buttermilk and cottage cheese; homogenizing pressure, temperature of pasteurization, feathering, sediment and whipping qualities of homogenized cream; and sediment and sweetening problems of chocolate milk. F.J.D.

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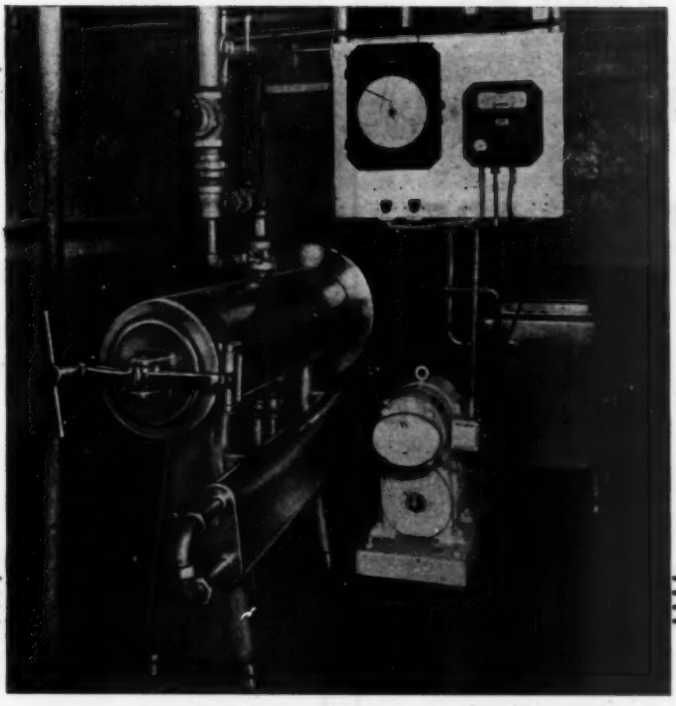

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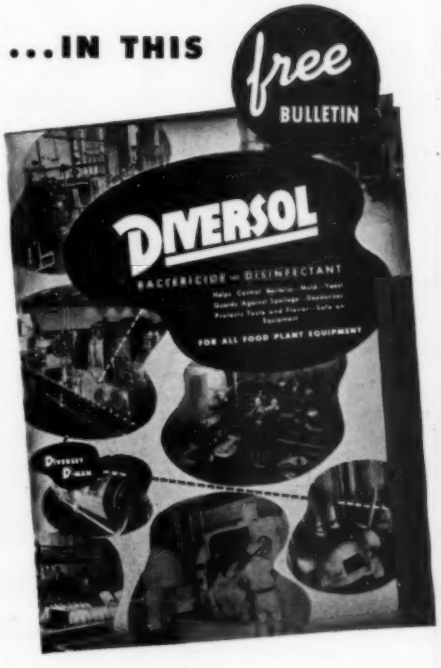

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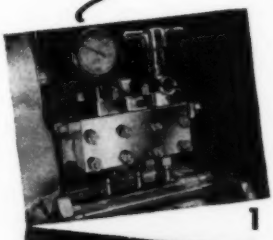

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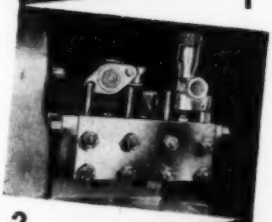

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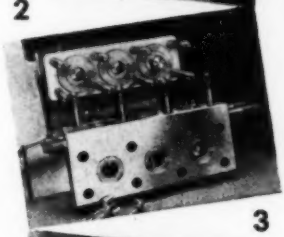

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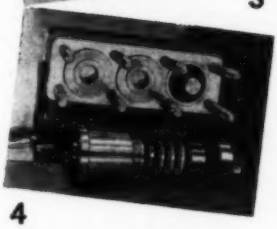

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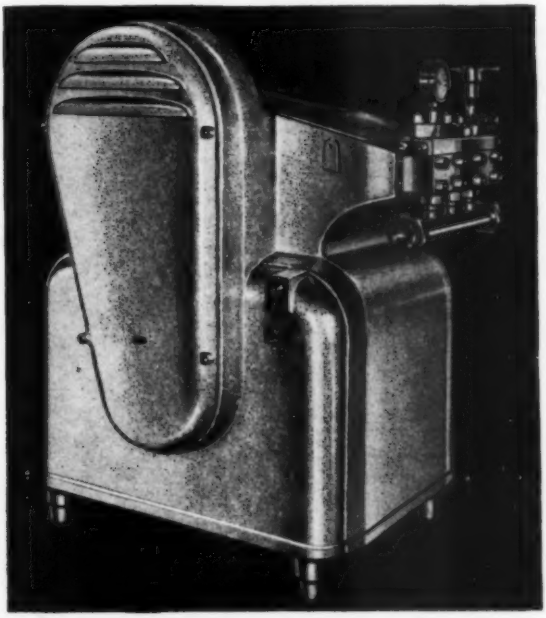

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